Exhibit A

JOURNAL OF INTERFERON AND CYTOKINE RESEARCH 19:661–669 (1999)
Mary Ann Liebert, Inc.

Early Expression of Proinflammatory Cytokines Interleukin-1 and Tumor Necrosis Factor-α after Corneal Transplantation

SUNING ZHU,[1] IVA DEKARIS,[1] GERNOT DUNCKER, and M. REZA DANA

ABSTRACT

This study's aim was to determine the early postoperative expression of proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) by corneal grafts. BALB/c ($n = 90$) and C57BL/6 ($n = 90$) murine recipients were grafted with donor corneas from either syngeneic or allogeneic mice. At 7 and 14 days after surgery, corneal grafts were excised and the recipient rims separated from the donor tissue. Corneal segments were cultured and assayed for cytokines by enzyme-linked immunosorbent assay (ELISA). There was profound upregulation in expression of both IL-1α and TNF-α after corneal transplantation. Among both low-rejecting BALB/c and high-rejecting C57BL/6 hosts, levels of IL-1α were significantly ($p < 0.01$) more marked in allogeneic as compared to syngeneic grafts. TNF-α overexpression was similarly more marked in allogeneic as compared to syngeneic grafts in both BALB/c and C57BL/6 hosts, although the difference was generally more marked among high-rejecting C57BL/6 recipients. In the case of both IL-1α and TNF-α, the principal source of cytokine expression in the transplanted tissue was the recipient rim. There is significant overexpression of both IL-1α and TNF-α during the first 2 weeks after transplantation in both syngeneic and allogeneic orthotopic corneal grafts. However, whereas in syngeneic grafts cytokine expression generally decreases after the first postoperative week, significantly elevated cytokine levels are sustained in allogeneic grafts, implicating IL-1 and TNF-α as mediators of the alloimmune response in corneal transplantation.

INTRODUCTION

CYTOKINES ARE SHORT-RANGED PROTEINS that may be active even at very low concentrations. Hence, small changes in the level of their expression may mediate profound changes in the immunoinflammatory response to antigenic (allogeneic) stimuli.[1] A number of cytokines have been detected in the eyes of patients with ocular inflammation, and experimental studies have shown that intraocular injection of specific cytokines can lead to uveitis, thereby establishing the link between the biological function of cytokines and the pathogenesis of ocular inflammation.[1,2]

Corneal transplantation (keratoplasty) is the most common and successful form of organ transplantation, with a survival rate in uncomplicated cases of approximately 90%.[3] This generally high success rate has been related to specific features of the corneal microenvironment, including its avascularity, low constitutive expression of major histocompatibility complex (MHC) antigens, absence of centrally situated antigen-presenting cells, local production of immunosuppressive cytokines, and expression of Fas ligand, all of which may alter both the induction and expression of alloimmunity.[4–7] However, in spite of the relative 'immune privilege' of corneal grafts, a significant number of allografts still suffer from rejection, and this is particularly the case in eyes with concurrent inflammatory disease where the rejection rate may well exceed 50%.[3]

Recent studies have implicated proinflammatory cytokines as mediators of transplant rejection. Studies of nonocular allografts, such as heart, liver, and kidney, have shown that interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) can act as mediators of the alloimmune response.[8–13] Although the molecular mechanisms of ocular alloimmunity remain poorly understood, there is rising evidence that proinflammatory cytokines IL-1 and TNF-α are involved in mediating the immune response in corneal transplantation as well.[14–17] Although the expression of these cytokines in rejecting corneal grafts is being increasingly appreciated,[15,16] other experimental data are revealing that, in addition to the well-known ocular risk factors for corneal allograft rejection,[3,18,19] there are appreciable variations in allograft survival rates among different strains of the same species, even in the setting of identical degrees of allodisparity.[20]

Laboratory of Immunology, Schepens Eye Research Institute, Harvard Medical School, Boston, MA 02114.
[1]Both authors contributed equally to this study.

The finding of significant differences in corneal graft survival in (high-rejecting C57BL/6 vs. low-rejecting BALB/c) murine strains provides a convenient experimental model for the analysis of cellular and molecular factors that could bias the immune response in favor of rejection, or survival. In the present study, we sought to determine the early expression of proinflammatory cytokines TNF-α, IL-1α, and IL-1β in response to corneal transplants in low-rejecting BALB/c and high-rejecting C57BL/6 hosts. Our data suggest that IL-1α and TNF-α are profoundly overexpressed after allogeneic transplantation regardless of the host background. However, whereas expression of both cytokines peak very early (1 week) among low-reject-

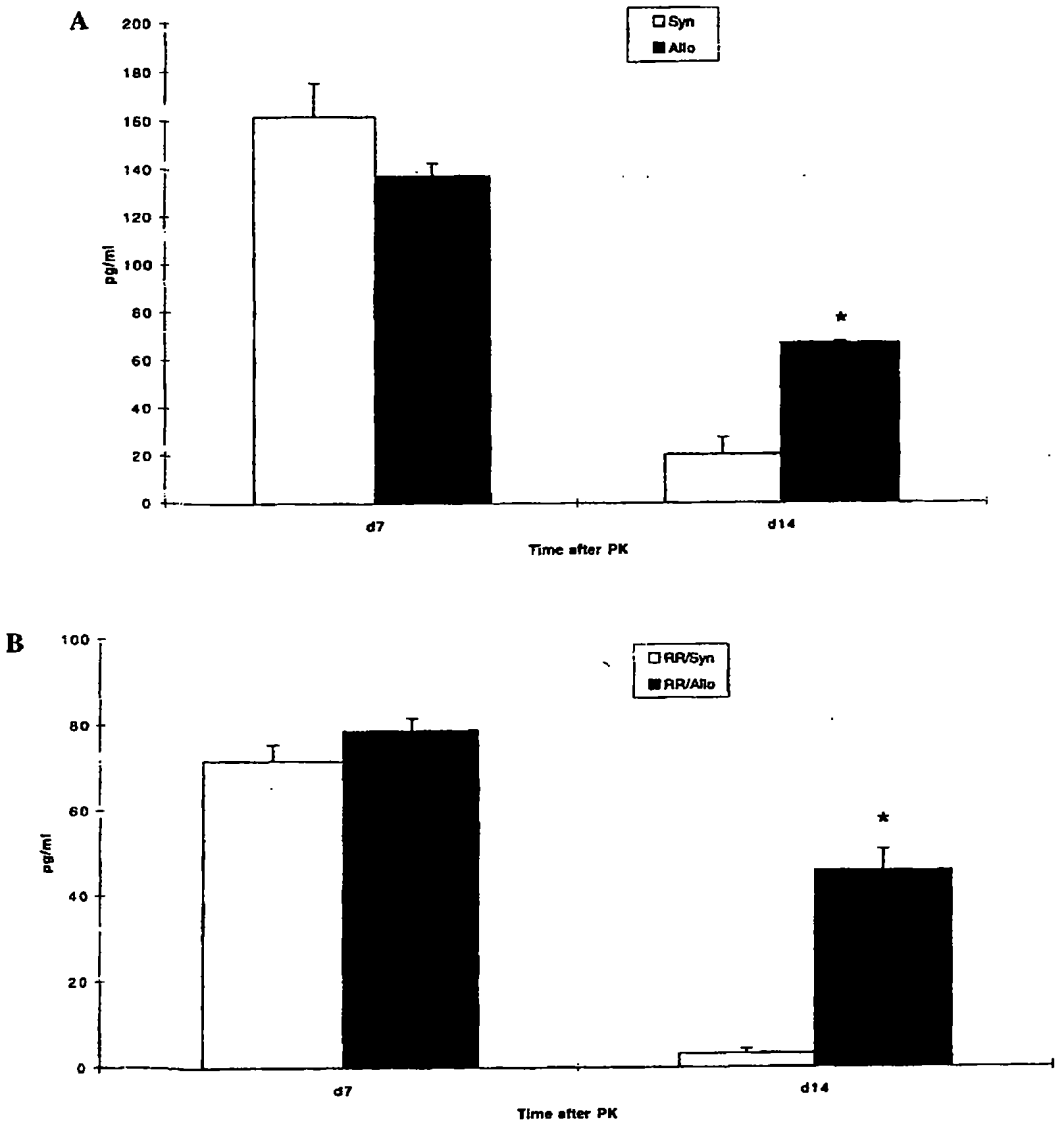


FIG. 1. IL-1α expression after corneal transplantation in low-rejecting BALB/c hosts: whole cornea (A), and recipient rim (RR) alone (B). Cytokine levels peak at day 7 in both allogeneic and syngeneic grafts. By day 14, IL-1α levels in syngeneic controls return to near basal levels, whereas levels remain significantly elevated in allografts ($p < 0.01$). The principal source of cytokine expression in allografts is the host rim (B), where at day 14 there is marked difference between IL-1α levels expressed by allogeneic as compared to syngeneic grafts ($p < 0.01$).

ing BALB/c hosts, there is sustained overexpression of both cytokines among high-rejecting C57BL/6 recipients of orthotopic allogeneic corneal grafts. In the aggregate, the data suggest that IL-1α and TNF-α expression is associated with the alloimmune response to corneal transplants.

MATERIALS AND METHODS

Mice and anesthesia

Eight- to 10-week-old BALB/c and C57BL/6 mice were purchased from Taconic Farms, Inc. (Germantown, NY). All ani-

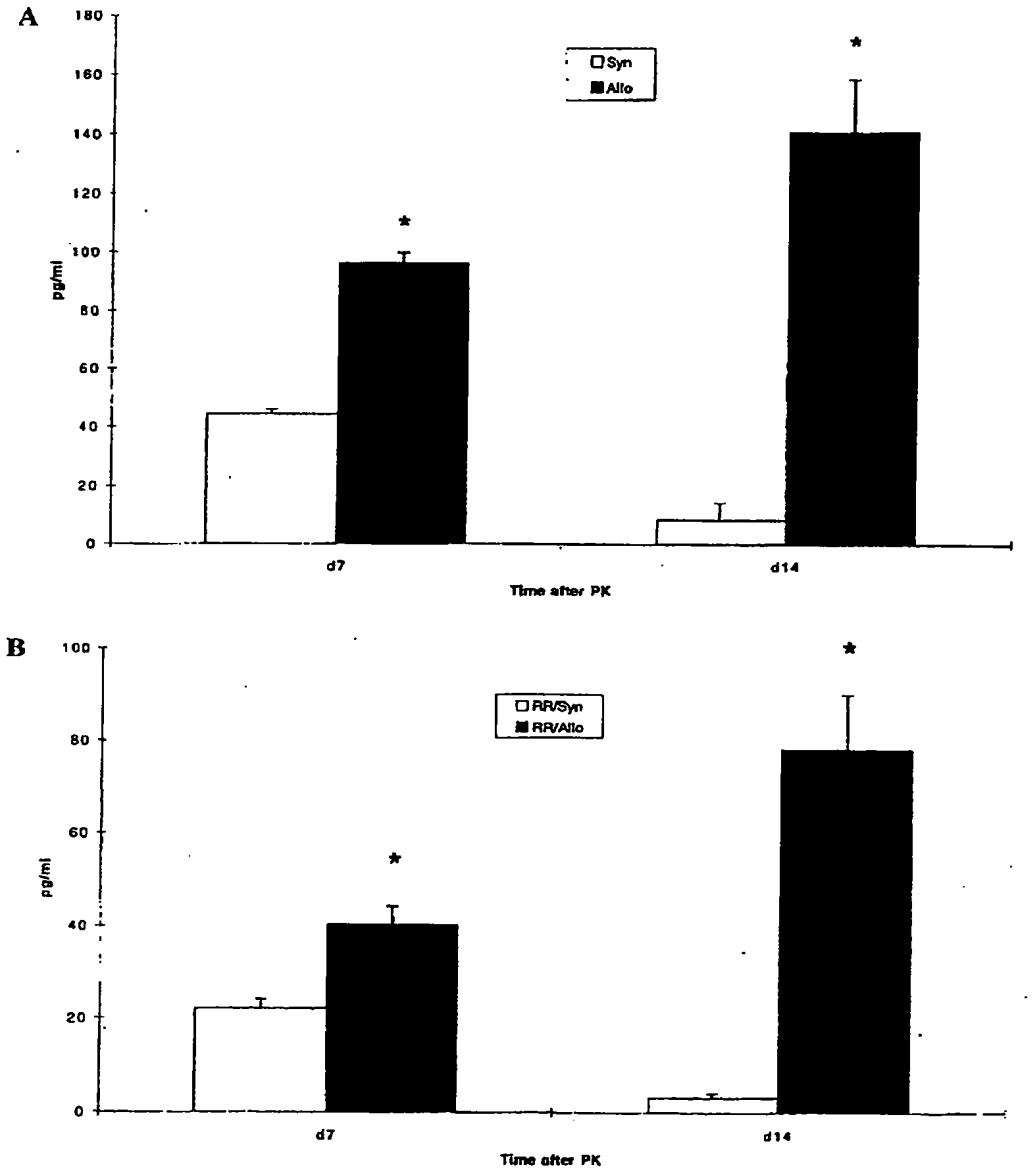


FIG. 2. IL-1α secretion after corneal transplantation in high-rejecting C57BL/6 hosts: whole cornea (A), and recipient rim (RR) alone (B). There is profound increase ($p < 0.01$) in levels of cytokine in both syngeneic and allogeneic grafts at day 7, which increase further in allografts by day 14 (A). There is significant difference in cytokine expression between allogeneic and syngeneic grafts at both day 7 ($p < 0.05$) and day 14 ($p < 0.01$), whether analyzing total corneal expression (A) or contribution by the host rim alone (B).

mals were treated according to the Statement for the Use of Animals in Ophthalmic and Vision Research by the Association for Research in Vision and Ophthalmology. Each animal was deeply anesthetized with an intramuscular injection of 3–4 mg of ketamine and 0.1 mg of xylazine prior to all surgical procedures.

Corneal transplantation

Corneal transplantation was performed from fully mismatched (MHC and multiple minor H disparate) C57BL/6 donors into BALB/c eyes ($n = 60$), and from BALB/c donors into C57BL/6 eyes ($n = 60$) as previously described.[14] Syngeneic grafts ($n = 30$ per strain) served as controls. Briefly, the central 2 mm of donor cornea was trephined and grafted into the recipient bed (2 mm) and sutured with eight interrupted 11-0 nylon sutures (Sharpoint, Vanguard, Houston, TX). Antibiotic ointment was applied and the lids were shut for 48 h with an 8-0 nylon tarsorrhaply. All graft sutures were removed on day 7.

Cytokine detection

At different time points following surgery (7 and 14 days), animals were sacrificed and the eyes were enucleated. Whole corneas, containing the donor button and recipient rim, were excised under the microscope and the recipient rims dissected from the donor button with minimal manipulation. Each corneal section, consisting of the donor button alone or the recipient rim, was cultured separately. Each test sample consisted of five corneas. Syngeneic grafts served as controls for the alloimmune response, and normal corneas were used as controls to determine the expression level of cytokines in unoperated tissue. Corneas were placed in serum-free RPMI 1640 medium (GIBCO-BRL, Gaithersburg, MD) and supplemented with Fungi-Bact Solution (Irvine Scientific, Santa Ana, CA) and assayed with commercially available IL-1α, IL-1β, and TNF-α ELISA kits (R & D Systems, Minneapolis, MN), as previously described.[21] Briefly, corneal samples were incubated in 120 μl of medium/well at 37°C under 5% CO_2 for 15 h, spun at 1,000 rpm for 5 min, after which cytokine assays were performed by enzyme-linked immunosorbent assay (ELISA) with 100 μl of the supernatant. The sensitivity of the ELISA was <3 pg/ml and <5.1 pg/ml for IL-1 and TNF-α, respectively. All experiments were repeated at least once, and statistical analysis was performed by the Student's t-test.

RESULTS

IL-1 secretion after corneal transplantation in low-rejecting BALB/c hosts

Transplanted and normal unoperated corneas were measured by ELISA. There was no detectable IL-1α expression in the unoperated corneas. In syngeneic controls, IL-1α secretion peaked at 7 days following corneal transplantation to 162 pg/ml, and dropped significantly close to basal levels (20 pg/ml) by 2 weeks after surgery (Fig. 1A). In corneal allografts, levels of IL-1α also peaked at day 7 but unlike syngeneic grafts, its expression at 2 weeks was sustained at significantly elevated levels (67 pg/ml) compared to syngeneic or unoperated controls ($p < 0.01$). In all allogeneic transplants, the majority of the IL-1α was secreted by the recipient rim of the graft (Fig. 1B) where the difference between cytokine expression by allogeneic and syngeneic controls at day 14 was particularly striking ($p < 0.01$). IL-1β was undetectable in both normal and transplanted syngeneic or allogeneic grafts at all time points assayed.

IL-1 secretion after corneal transplantation in high-rejecting C57BL/6 hosts

IL-1α production in both syngeneic and allografted corneas increased significantly as compared to the undetectable levels of normal corneal tissue ($p < 0.01$; Fig. 2A). In syngeneic grafts, IL-1α production returned to almost constitutive levels by day 14 after grafting, in contrast to allogeneic grafts in which IL-1α expression continued to increase for the duration of follow-up. Moreover, allograft levels of IL-1α were significantly higher as compared to syngeneic grafts at both the 1- (96 vs. 44 pg/ml; $p < 0.05$) and 2-week (140 vs. 8 pg/ml; $p < 0.01$) postoperative periods (Fig. 2A). Similar to results in the low-rejecting BALB/c hosts, a significant portion of the secreted IL-1α after allogeneic corneal transplantation was expressed by the recipient rim (Fig. 2B). As in the case of BALB/c mice, there was no detectable IL-1β in any of the corneal samples studied.

TNF-α secretion after corneal transplantation in low-rejecting BALB/c hosts

Normal BALB/c corneal tissue expressed very low levels (10 pg/ml) of TNF-α that profoundly increased following both syngeneic and allogeneic corneal transplantation ($p < 0.01$; Fig. 3A). Similar to IL-1α, peak secretion of TNF-α in both syngeneic (52 pg/ml) and allogeneic (95 pg/ml) grafts was at day 7 postoperatively. However, unlike IL-1α, for which levels in syngeneic grafts largely normalized by day 14, significantly elevated levels of cytokine were documented throughout the two postoperative weeks. The level of TNF-α at day 7, particularly that secreted by the recipient rim (Fig. 3B) was significantly ($p < 0.05$) higher in allogeneic (74 pg/ml) as compared to syngeneic transplants (28 pg/ml). Similar to IL-1α, the principal source of the secreted TNF-α after corneal transplantation was expression by the host rim, and this was particularly evident in the allogeneic graft setting (Fig. 3B).

TNF-α secretion after corneal transplantation in high-rejecting C57BL/6 hosts

The level of unstimulated TNF-α secretion in unoperated normal C57BL/6 corneas was similar to that observed in the normal BALB/c corneas (9 pg/ml). Seven days after corneal transplantation, there was a moderate increase in TNF-α secretion in allografts. The three-fold (27 pg/ml) increase in the expression of TNF-α in allogeneic grafts in the first week was significantly ($p < 0.05$) higher than that observed in normal unstimulated corneas or syngeneic grafts (Fig. 4A). By day 14, a profound (eight-fold) overexpression of TNF-α (70 pg/ml) was observed in allogeneic, but not syngeneic, grafts ($p < 0.01$). Similar to our other observations, the majority of the secreted TNF-α was by the recipient rim of the transplant (Fig. 4B).

DISCUSSION

Corneal graft rejection remains by far the leading cause of irreversible transplant failure in humans.[3] Hence, investigation into the molecular mechanisms involved in the induction of allosensitization and expression of graft rejection is a priority in corneal transplantation research. Recent studies in experimental models of transplantation have underscored the

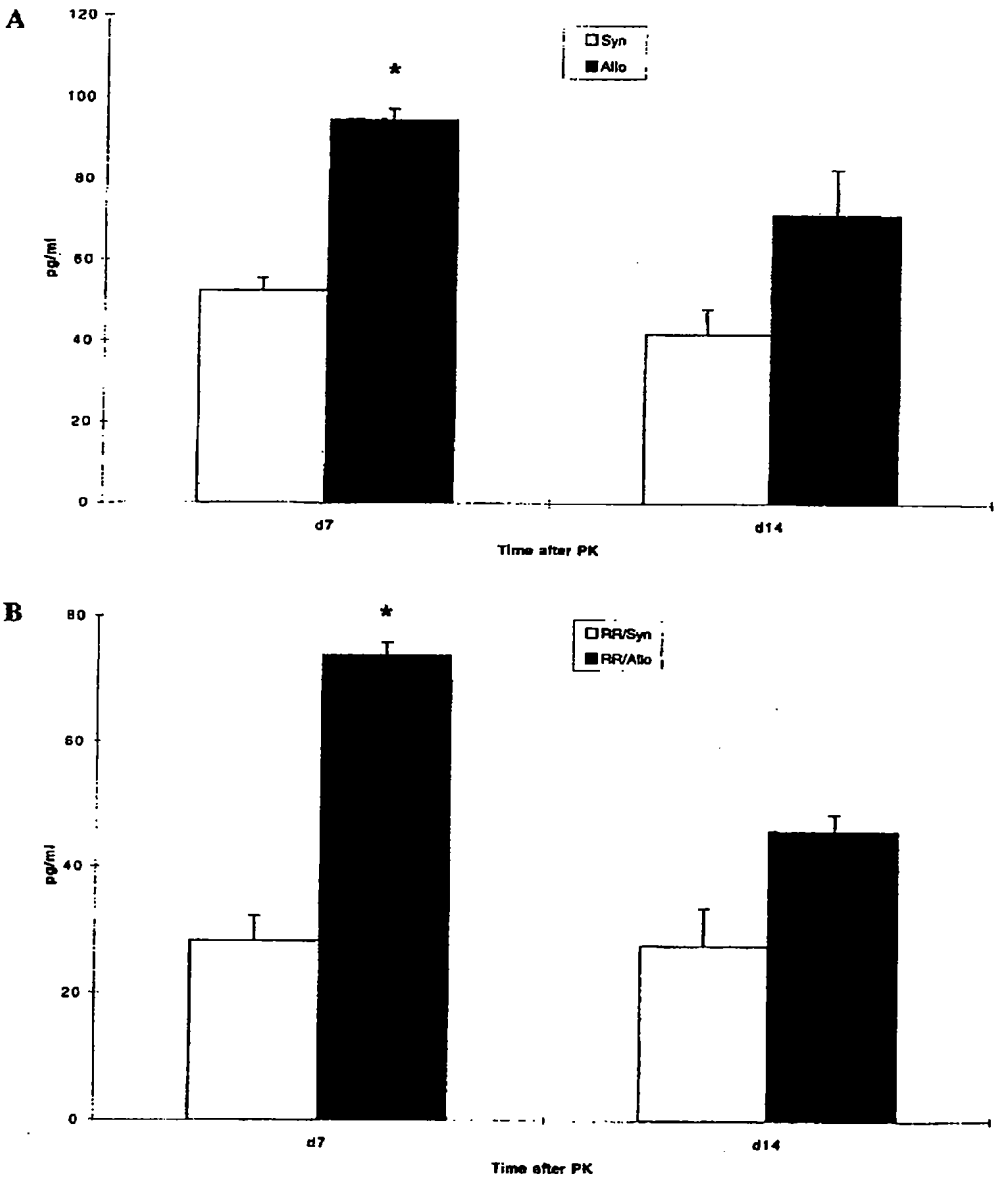


FIG. 3. TNF-α secretion after corneal transplantation in low-rejecting BALB/c hosts: whole cornea (A), and recipient rim (RR) alone (B). Transplantation led to significant overexpression of TNF-α in both syngeneic and allogeneic grafts (A). Peak secretion occurred at day 7, but levels remained profoundly elevated for the duration of follow-up ($p < 0.01$). TNF-α expression in allografts at day 7 was significantly ($p < 0.05$) higher than that in syngeneic grafts, and this difference was particularly evident in the recipient rim (B).

role of proinflammatory cytokines in regulation of the alloimmune response.[8-13] These cytokines have multiple, and often overlapping, activities that generally lead to amplification of immune and inflammatory responses through induction of chemokines,[22] activation of antigen-presenting cells (APCs),[23] and increased expression of adhesion and costimulatory molecules.[24]

There is some evidence that relates IL-1 and TNF-α activity to corneal allograft rejection. In a murine model of corneal transplantation, it has been shown that local suppression of IL-1 receptor activity by topical IL-1 receptor antagonist can be effective in promoting corneal allograft survival,[14] in part through suppressing APC activity and allosensitization.[25] Similarly, in relation to TNF-α, Torres and co-workers have shown

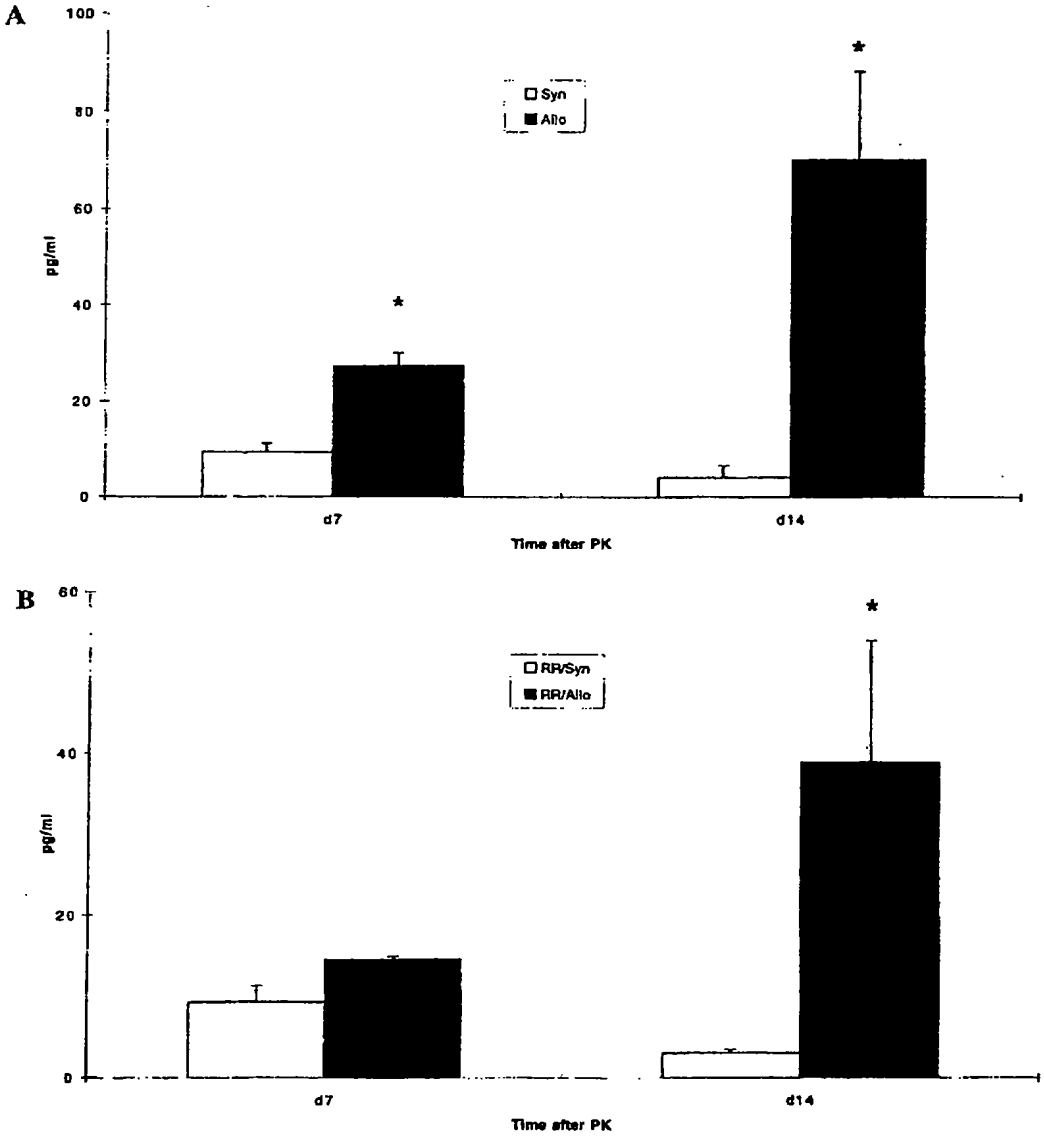


FIG. 4. TNF-α secretion after corneal transplantation in high-rejecting C57BL/6 hosts: whole cornea (A), and recipient rim (RR) alone (B). There is a three-fold increase in cytokine expression in allografts by day 7 ($p < 0.05$). While TNF-α levels in syngeneic grafts remain depressed at basal levels, there is increasing expression in allografts by day 14 ($p < 0.01$). The principal source of TNF-α in the allografts is the recipient rim (B).

that mRNA levels for TNF-α are significantly higher in rejecting corneal allografts as compared to autografts,[15] and Larkin and co-workers have shown high TNF-α expression by aggregated mononuclear cells on the graft endothelium of rejected corneal allografts in rats,[16] providing indirect evidence for the role of these cytokines in corneal transplant rejection. In addition, Pleyer and co-workers have determined that serum expression of TNF-α is considerably elevated in rat corneal graft recipients rejecting their transplants as compared to controls without grafts, or rats without allograft rejection,[17] suggesting that systemic TNF-α levels are associated with the expression of alloimmunity in corneal transplantation.

While these studies have implicated the role of IL-1 and TNF-α in corneal graft rejection, to date the expression of these cytokines in the early post-keratoplasty period has not been systematically studied, nor has the expression of these cytokines in low-rejecting vs. high rejecting hosts been evaluated. In this report, we have examined the early (induction phase) expression of IL-1 and TNF-α in a standardized murine model of corneal transplantation to determine the expression of these proinflammatory cytokines prior to graft rejection. In this model of transplantation, graft rejection typically occurs after 2–3 weeks and is characterized by profound stromal edema of the graft associated with a mixed leukocyte infiltrate.[14,26] We elected to study cytokine expression in the early postoperative period because we were interested in local cytokine production prior to full expression of the immune response, when infiltrating effector cells contribute significantly to the local cytokine milieu. Recent experiments have shown that the rate of transplant rejection survival can be affected by the strain of recipient animals even given the same degree of allodisparity.[20] Accordingly, fully mismatched corneal grafts from BALB/c into C57BL/6 mice experience higher rates of rejection (~90%) compared to rejection rates of C57BL/6 allografts performed in BALB/c mice (~50%). In this series of experiments, we hypothesized that there is a significant upregulation in the expression of both TNF-α and IL-1α after orthotopic corneal allotransplantation; and moreover, that the poorer survival rate of grafts in C57BL/6 hosts can be explained, at least in part, by differences in the postoperative expression of these proinflammatory cytokines among these two recipient strains.

Our results may be summarized as follows: (i) there is a significant overexpression of both IL-1α and TNF-α after corneal transplantation; (ii) the enhanced expression of these cytokines is particularly evident in allogeneic grafts even prior to graft rejection; (iii) there is sustained and increasing overexpression of IL-1α and TNF-α in high-rejecting C57BL/6 hosts of allogeneic grafts compared to levels in BALB/c hosts or syngeneic grafts in which cytokine levels decrease after peaking at one week postoperatively; and (iv) the principal source of IL-1 and TNF-α in the corneal transplant is from the recipient rim.

Several lines of experimental data in both humans and rodents have shown that corneal cells have the endogenous potential to produce proinflammatory cytokines.[27–29] In this respect, our data confirm the laboratory evidence that traumatic and inflammatory insults to the cornea can induce overexpression of TNF-α[30] and IL-1α.[21,29,31] However, the sustained high expression of IL-1α in our model cannot be explained entirely by the trauma of the transplant surgery, as reflected by the near-normalization of IL-1α levels in syngeneic, but not allogeneic, grafts. Hence our data suggest that the high expression of IL-1α is particularly relevant to the allogeneic corneal graft setting. In contrast, levels of IL-1β are undetectable in both syngeneic and allogeneic grafts. This contrasts with other laboratory data quantifying IL-1 levels after alkali burns[29] or induction of inflammatory corneal neovascularization,[21] where significant expression of IL-1β has also been documented. However, it has been shown that the expression of IL-1β is evident only in the first 48 h after induction of corneal neovascularization and is undetectable thereafter.[21] Hence, our failure to document IL-1β expression after transplantation could be a reflection of our choice of time points for assaying cytokine levels, or indicative of selective induction of IL-1 isoforms early after corneal transplantation.

Our data suggest that the principal source of expressed cytokine is the recipient rim of the transplant. Because our protocol was based on culturing the corneal tissue, and not on direct *in situ* analysis for the source of cytokine, our data cannot determine to what extent the secreted cytokines are from the corneal cells or from leukocytes infiltrating the graft from the vascularized limbal area. However, the cytokines in these experiments were assayed well before the peak infiltration of corneal grafts by host effector and inflammatory cells. Moreover, because resident corneal, in particular epithelial, cells are capable of profound expression of proinflammatory cytokines under the appropriate setting, and because mesenchymal cells primarily release IL-1α, as opposed to IL-1β, which is chiefly expressed by bone marrow-derived cells, it is likely that a major source of these cytokines is the resident corneal cells.

Sano and co-workers have recently reported increased IL-1α and TNF-α production from grafted corneas into BALB/c recipients, but were not able to find any difference in cytokine levels between syngeneic and allogeneic grafts at 1 week after surgery.[32] For IL-1α, but not TNF-α, our data are in accordance with their results at the 1-week period when our data in BALB/c recipients also demonstrate similar cytokine expression among syngeneic and allogeneic grafts. However, direct comparison of our data with their work is difficult due to differences in assaying technique, tissue analyzed (Sano and coworkers evaluated 4 mm of excised tissue, which included the corneoscleral limbus), strains studied (they only evaluated BALB/c hosts), and time points studied (they only evaluated one early time point after transplantation).

The increased and sustained overexpression of IL-1α and TNF-α in allogeneic grafts, particularly in high-rejecting C57BL/6 hosts, strongly implicates these cytokines in the early alloimmune response to corneal grafts. However, we do not feel that our data necessarily provide definitive proof for the hypothesis that the high rejection rate of corneal grafts in C57BL/6 hosts is due to the differential expression of these cytokines at the site of the graft early after transplantation. For example, in spite of a modest increased expression of IL-1α in C57BL/6 (compared to BALB/c) hosts, particularly at 2 weeks after transplantation, our data suggest that overall expression of TNF-α is in fact more marked in BALB/c hosts. Notwithstanding the above, the kinetics of cytokine expression after corneal transplantation differ significantly between the two recipient strains, for whereas the cytokine expression peaks early and then decreases in BALB/c hosts, the expression in C57BL/6 recipients shows a steady and sustained increase with time. Moreover, be-

cause we focused on the early (first 2 week) production of IL-1 and TNF-α after corneal transplantation prior to any documented rejection, we cannot rule out that sustained upregulation in expression of these cytokines could be causally related to the high rate of subsequent allograft rejection in C57BL/6 recipients. Finally, although our experiments do not address this hypothesis, it is intriguing to postulate that the distinct expression of cytokines among the two strains (*e.g.*, Th1-polarized C57BL/6 vs. Th2-polarized BALB/c) reflects their differential requirements for inducing alloimmune T cell responses. For example, it has recently been shown that the requirements for IL-1α and TNF-α for optimal priming of ovalbumin-specific Th1 cells differ considerably between BALB/c and C57BL/6 mice.[33]

In summary, as the first report focusing on the early and strain-specific expression of proinflammatory cytokines after orthotopic corneal transplantation, our data suggest that IL-1α and TNF-α are associated with the alloimmune response to corneal allografts. It is anticipated that better understanding of the early molecular events following corneal transplantation and determination of the critical mediators of the alloimmune response will one day lead to better protection of corneal grafts, the most common form of allotransplantation, from irreversible rejection.

ACKNOWLEDGMENTS

This work was supported by NIH-EY00363, Eye Bank Association of America, and Fight For Sight (M.R.D.).

REFERENCES

1. KIJLSTRA, A. (1994). The role of cytokines in ocular inflammation. Br. J. Ophthalmol. **78**, 885–886.
2. DE VOS, A.F., HOEKZEMA, R., and KIJLSTRA, A. (1992). Cytokines and uveitis review. Curr. Eye Res. **11**, 581–597.
3. THE COLLABORATIVE CORNEAL TRANSPLANTATION STUDIES RESEARCH GROUP. (1992). The collaborative corneal transplantation studies (CCTS): effectiveness of histocompatibility matching in high-risk corneal transplantation. Arch. Ophthalmol. **110**, 1392–1403.
4. STREILEIN, J.W., WILBANKS, G.A., and COUSINS, S.W. (1992). Immunoregulatory mechanisms of the eye. J. Neuroimmunol. **39**, 185–200.
5. STREILEIN, J.W., WILBANKS, G.A., TAYLOR, A.W., and COUSINS, S.W. (1992). Eye-derived cytokines and the immunosuppressive intraocular microenvironment: A review. Curr. Eye Res. **11**, 41–47.
6. NIEDERKORN, J.Y. (1990). Immune privilege and immune regulation in the eye. Adv. Immunol. **48**, 191–194.
7. GRIFFITH, T.S., BRUNNER, T., FLETCHER, S.M., GREEN, D.R., and FERGUSON, T.A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. Science **270**, 1189–1192.
8. BUCHWALD, S., FRIEMEL, H., PLANTIKOW, A., HUDEMANN, B., BAST, R., and TEMPLIN, R. (1990). Clinical value of interleukin-1 and interleukin-2-determinations in patients after kidney transplantation. Allerg. Immunol. **36**, 137.
9. TAKASU, S., SAKAGAMI, K., and ORITA, K. (1989). A new immunosuppressant, 15-deoxyspergualin, inhibits production of IL-1 from isolated hepatic sinusoidal lining cells in swine liver transplantation. Transpl. Proc. **21**, 1081–1084.
10. TILG, H., VOGEL, W., and AULITZKY, W.E. (1990). Evaluation of cytokines and cytokine-induced secondary messages in sera of patients after liver transplantation. Transplantation **49**, 1074–1080.
11. IMAGAWA, D.K., MILLIS, J.M., OLTHOFF, K.M., DERUS, L.J., CHIA, D., SUGICH, L.R., OZAWA, M., DEMPSEY, R.A., IEAK, I.Y., and LEVY, P.J. (1990). The role of tumor necrosis factor in allograft rejection. I. Evidence that elevated levels of tumor necrosis factor-alpha predict rejection following orthotopic liver transplantation. Transpl. Proc. **50**, 219–225.
12. PIZZARO, T.T., MALINOWSKA, K., KOVACS, E.J., *et al.* (1993). Induction of TNF alpha and TNF beta gene expression in rat cardiac transplant during allograft rejection. Transplantation **56**, 399–404.
13. KUTUCKLER, N., SHENTON, B.K., CLARK, K., *et al.* (1995). Renal allograft rejection: the temporal relationship and predictive value of plasma TNF (alpha and beta), IFN-gamma and soluble ICAM-1. Transpl. Int. **8**, 45–50.
14. DANA, M.R., YAMADA, J., and STREILEIN, J.W. (1997). Topical interleukin 1 receptor antagonist promotes corneal transplant survival. Transplantation **63**, 1501–1507.
15. TORRES, P.F., DE VOS, A.F., VAN DER GAAG, R., MARTINS, B., and KIJLSTRA, A. (1996). Cytokine mRNA expression during experimental corneal allograft rejection. Exp. Eye Res. **63**, 453–461.
16. LARKIN, D.F., CALDER, V.L., and LIGHTMAN, S.L. (1997). Identification and characterization of cells infiltrating the graft and aqueous humor in rat corneal allograft rejection. Clin. Exp. Immunol. **107**, 381–391.
17. PLEYER, U., MILANI, J.K., RUCKERT, D., *et al.* (1997). Determination of serum tumor necrosis factor alpha in corneal allografts. Ocul. Immunol. Inflamm. **5**, 149–155.
18. SANO, Y., KSANDER, B.R., and STREILEIN, J.W. (1995). Fate of orthotopic corneal allografts in eyes that cannot support anterior chamber-associated immune deviation induction. Invest. Ophthalmol. Vis. Sci. **36**, 2176–2185.
19. SANO, Y., KSANDER, B.R., and STREILEIN, J.W. (1997). Murine orthotopic corneal transplantation in high-risk eyes: rejection is dictated by weak rather than strong alloantigens. Invest. Ophthalmol. Vis. Sci. **38**, 1130–1138.
20. YAMADA, J., and STREILEIN, J.W. (1998). Fate of orthotopic corneal allografts in C57BL/6 mice. Transpl. Immunol. **6**, 1–8.
21. DANA, M.R., ZHU, S.N., and YAMADA, J. (1998). Topical modulation of interleukin-1 activity in corneal neovascularization. Cornea **17**, 403–409.
22. LUSTER, A.D. (1998). Chemokines-chemotactic cytokines that mediate inflammation. N. Engl. J. Med. **338**, 436–452.
23. DINARELLO, C.A., and WOLFF, S.M. (1993). The role of interleukin-1 in disease. N. Engl. J. Med. **328**, 106–113.
24. SPRINGER, T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell **76**, 301–314.
25. YAMADA, J., DANA, M.R., *et al.* (1998). Interleukin 1 receptor antagonist suppresses allosensitization in corneal transplantation. Arch. Ophthalmol. **116**, 1351–1357.
26. SONODA, Y., and STREILEIN, J.W. (1992). Orthotopic corneal transplantation in mice: evidence that the immunogenetic rules of rejection do not apply. Transplantation **54**, 694–704.
27. WILSON, S.E., HE, Y.G., and LLOYD, S.A. (1992). EGF, EGF receptor, basic FGF, TGF beta-1 and IL-1 alpha mRNA in human corneal epithelial cells and stromal fibroblasts. Invest. Ophthalmol. Vis. Sci. **33**, 1756–1765.
28. PLANCK, S.R., RICH, L.F., ANSEL, J.C., HUANG, X.N., and

ROSENBAUM, J.T. (1997). Trauma and alkali burns induce distinct pattern of cytokine gene expression in the rat cornea. Ocul. Immunol. Inflamm. 5, 95–100.
29. SOTOZONO, C., HE, J., MATSUMOTO, Y., KITA, M., IMANISHI, J., and KINOSHITA, S. (1997). Cytokine expression in the alkali-burned cornea. Curr. Eye Res. 16, 670–676.
30. SEKINE-OKANO, M., LUCAS, R., RUNGGER, D., DE KESSEL, T., GRAU, G.E., LEUENBERGER, P.M., and RUNGGER-BRANDLE, E. (1996). Expression and release of tumor necrosis factor-alpha by explants of mouse cornea. Invest. Ophthalmol. Vis. Sci. 37, 1302–1310.
31. LAUSCH, R.N., CHEN, S.H., TUMPEY, T.M., SU, Z.H., and OAKES, J.E. (1996). Early cytokine synthesis in the excised mouse cornea. J. Interferon Cytokine Res. 16, 35–40.
32. SANO, Y., OSAWA, H., SOTOZONO, C., and KINOSHITA, S. (1998). Cytokine expression during orthotopic corneal allograft rejection in mice. Invest. Ophthalmol. Vis. Sci. 39, 1953–1957.
33. SHIBUYA, K., ROBINSON, D., ZONIN, F., HARTLEY, S.B., MACATONIA, S.E., COMOZA, C., HUNTER, C.A., MURPHY, K.M., and O'GARRA, A. (1998). IL-1α and TNFα are required for IL-12-induced development of Th1 cells producing high levels of IFN-γ in BALB/c but not C57BL/6 mice. J. Immunol. 160, 1708–1716.

Address reprint requests to:
Dr. M. Reza Dana
Laboratory of Immunology
Schepens Eye Research Institute
Harvard Medical School
20 Staniford Street
Boston, MA 02114

Tel: 617-912-7404
Fax: 617-912-0117
E-mail: dana@vision.eri.harvard.edu

Received 11 February 1999/Accepted 22 February 1999

Journal of Interferon and Cytokine Research

The Official Publication of the
International Society for Interferon and Cytokine Research

Editorial Board

General Information

JOURNAL OF INTERFERON AND CYTOKINE RESEARCH is the primary source for original publications on all the varied aspects of research on the interferon and cytokine systems, including all basic and clinical studies.

JOURNAL OF INTERFERON AND CYTOKINE RESEARCH (ISSN: 1079-9907) is published 12 times a year (monthly) by Mary Ann Liebert, Inc., 2 Madison Avenue, Larchmont, NY 10538-1962. Periodicals postage paid at Larchmont, NY, and at additional mailing offices. Mailed in Canada under CPC IPM #1269755. **Postmaster:** Send address changes to *JOURNAL OF INTERFERON AND CYTOKINE RESEARCH*, c/o Subscription Department, Mary Ann Liebert, Inc., 2 Madison Avenue, Larchmont, NY 10538–1962.

Subscriptions should be addressed to the Publisher and are payable in advance. Rates for subscriptions are $752 per volume of 12 issues in the United States and Possessions, and $907 elsewhere. Subscriptions begin with the first issue of the current volume. Members of ISICR pay a reduced subscription rate of $110 in the United States and Canada, and $165 foreign. Bulk subscriptions are available upon request from the Publisher. No cancellations/refunds can be honored after publication of a volume's first issue.

Telephone: (914) 834-3100; fax: (914) 834-3582; e-mail: info@liebertpub.com on line: www.liebertpub.com

Business communications should be addressed to the Publisher.

Advertising inquiries should be addressed to Mary Ann Liebert, Inc., 2 Madison Avenue, Larchmont, NY 10538. (914) 834-3100. In Europe, contact Hilary Turnbull, imPRESS, 2 Penrith Avenue, Glasgow G46 6LU, Scotland, U.K. Telephone: +44.141.620.0106; fax: +44.141.620.0055; e-mail: hturnbull@impressmedia.com All advertisements are subject to approval by the Publisher.

Reprints, except special orders of 100 or more, are available from the authors. For permission to photocopy for internal purposes 24 copies or less, please request permission and pay the appropriate fee by contacting the Customer Relations Dept. of the Copyright Clearance Center, Inc., 22 Rosewood Drive, Danvers, MA 01923, tel: 978-750-8400, fax: 978-750-4470. If the number of copies of an article is 25 or higher, contact Reprint Sales Manager, Karen Ballen, at 914-834-3100 ext. 609 directly for options.

Manuscripts should be directed to the appropriate Editor-in-Chief. For INTERFERONS: **Philip I. Marcus, Department of Molecular and Cell Biology, University of Connecticut, 75 North Eagleville Road, Storrs, Connecticut 06269-3044.** For CYTOKINES: **Lawrence B. Lachman, Department of Bioimmunotherapy, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030.**

JOURNAL OF INTERFERON AND CYTOKINE RESEARCH is a **Journal Club** selection.

This Journal is indexed in *Index Medicus, Current Contents/Life Science, EMBASE/Excerpta Medica, Cambridge Scientific Abstracts, Reference Update, ISI/Biomed,* and *Scientific Citation Index.*

Please visit us on the web: www.liebertpub.com

Mary Ann Liebert, Inc. Publishers

Exhibit B

Crit Rev Ther Drug Carrier Syst. 1999;16(1):85-146.

Enhancement of ocular drug penetration.

Sasaki H, Yamamura K, Mukai T, Nishida K, Nakamura J, Nakashima M, Ichikawa M.

Department of Hospital Pharmacy, Nagasaki University School of Medicine, Japan.

Although new drugs have recently been developed within the field of ophthalmology, the eye's various defense mechanisms make it difficult to achieve an effective concentration of these drugs within the eye. Drugs administered systemically have poor access to the inside of the eye because of the blood-aqueous and blood-retinal barriers. And although topical instillation of drugs is very popular in ophthalmology, topically applied drugs are rapidly eliminated from the precorneal area. In addition, the cornea, considered a major pathway for ocular penetration of topically applied drugs, is an effective barrier to drug penetration, since the corneal epithelium has annular tight junctions (zonula occludens), which completely surround and effectively seal the superficial epithelial cells. Various drug-delivery systems have been developed to increase the topical bioavailability of ophthalmic drugs by enhancement of the ocular drug penetration. The first approach is to modify the physicochemical property of drugs by chemical and pharmaceutical means. An optimum promoiety can be covalently bound to a drug molecule to obtain a prodrug that can chemically or enzymatically be converted to the active parent drug, either within the cornea or after the corneal penetration. Along these same lines, the transient formation of a lipophilic ion pair by ionic bonding is also useful for improving ocular drug penetration. The second approach is to modify the integrity of the corneal epithelium transiently by coadministration of an amphiphilic substance or by chelating agents that act as drug-penetration enhancers. The third approach modifies the integrity of the corneal epithelium transiently by physical techniques including iontophoresis and phonophoresis. This paper reviews the absorption behavior and ocular membranes penetration of topically applied drugs, and the various approaches for enhancement of ocular drug penetration in the eye.

PMID: 10099899 [PubMed - indexed for MEDLINE]

Exhibit C

Cancer Metastasis Rev. 2001;20(1-2):123-32.

Tumor physiology and drug resistance.

Tannock IF.

Department of Medical Oncology, Princess Margaret Hospital and University of Toronto, ON, Canada.
ian.tannock@uhn.on.ca

Clinical resistance is usually assumed to be due to the initial presence or selection of drug-resistant cells in tumors. While important, it is suggested in this review that genetically-determined causes of cellular resistance represent but one cause (and possibly not the major cause) of effective clinical resistance of solid tumors. Factors that depend on tumor physiology, and on the microenvironment and three-dimensional structure of solid tumors, may have a profound influence on their sensitivity to anti-cancer drugs. Particular emphasis is placed on the limited penetration of some drugs from tumor blood vessels and on the repopulation of tumor cells between courses of chemotherapy as causes of clinical resistance. Both of these mechanisms are amenable to modulation to improve therapeutic index. Failure to recognize that clinical drug resistance cannot be explained entirely by mechanisms operative at the level of the single cell may lead to disappointing results in clinical trials such as, for example, clinical failure of the strategy of reversal of multidrug resistance.

PMID: 11831640 [PubMed - indexed for MEDLINE]

Exhibit D

Cancer Metastasis Rev. 1990 Nov;9(3):253-66.

Vascular and interstitial barriers to delivery of therapeutic agents in tumors.

Jain RK.

Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213-3890.

The efficacy in cancer treatment of novel therapeutic agents such as monoclonal antibodies, cytokines and effector cells has been limited by their inability to reach their target in vivo in adequate quantities. Molecular and cellular biology of neoplastic cells alone has failed to explain the nonuniform uptake of these agents. This is not surprising since a solid tumor in vivo is not just a collection of cancer cells. In fact, it consists of two extracellular compartments: vascular and interstitial. Since no blood-borne molecule or cell can reach cancer cells without passing through these compartments, the vascular and interstitial physiology of tumors has received considerable attention in recent years. Three physiological factors responsible for the poor localization of macromolecules in tumors have been identified: (i) heterogeneous blood supply, (ii) elevated interstitial pressure, and (iii) large transport distances in the interstitium. The first factor limits the delivery of blood-borne agents to well-perfused regions of a tumor; the second factor reduces extravasation of fluid and macromolecules in the high interstitial pressure regions and also leads to an experimentally verifiable, radially outward convection in the tumor periphery which opposes the inward diffusion; and the third factor increases the time required for slowly moving macromolecules to reach distal regions of a tumor. Binding of the molecule to an antigen further lowers the effective diffusion rate by reducing the amount of mobile molecule. Although the effector cells are capable of active migration, peculiarities of the tumor vasculature and interstitium may be also responsible for poor delivery of lymphokine activated killer cells and tumor infiltrating lymphocytes in solid tumors. Due to micro- and macroscopic heterogeneities in tumors, the relative magnitude of each of these physiological barriers would vary from one location to another and from one day to the next in the same tumor, and from one tumor to another. If the genetically engineered macromolecules and effector cells, as well as low molecular weight cytotoxic agents, are to fulfill their clinical promise, strategies must be developed to overcome or exploit these barriers. Some of these strategies are discussed, and situations wherein these barriers may not be a problem are outlined. Finally, some therapies where the tumor vasculature or the interstitium may be a target are pointed out.

PMID: 2292138 [PubMed - indexed for MEDLINE]

Exhibit E

Annu Rev Biomed Eng. 1999;1:241-63.

Transport of molecules, particles, and cells in solid tumors.

Jain RK.

Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, USA. jain@steele.mgh.harvard.edu

Extraordinary advances in molecular biology and biotechnology have led to the development of a vast number of therapeutic anti-cancer agents. To reach cancer cells in a tumor, a blood-borne therapeutic molecule, particle, or cell must make its way into the blood vessels of the tumor and across the vessel wall into the interstitium, which it then must migrate through. Unfortunately, tumors often develop in ways that hinder these steps. The goal of research in this area is to analyze each of these steps experimentally and theoretically and integrate the resulting information into a unified theoretical framework. This paradigm of analysis and synthesis has fostered a better understanding of physiological barriers in solid tumors and aided in the development of novel strategies to exploit and/or overcome these barriers for improved cancer detection and treatment.

PMID: 11701489 [PubMed - indexed for MEDLINE]

Exhibit F

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 8053
LÓPEZ DE SILANES *et al.*	Art Unit: 1618
Appl. No.: 10/565,885	Examiner: Skelding, Zachary S.
§ 371(c) Date: January 25, 2006	Atty. Docket: 2099.0080000/PAJ/LMB
For: **Administration of Anti-Cytokine $F(ab')_2$ Antibody Fragments**	

Declaration Under 37 C.F.R. § 1.132 of Dr. Jorge F. Paniagua-Solís

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, **Dr. Jorge F. Paniagua-Solís**, declare and state that:

1. I am a co-inventor of the above-captioned U.S. Patent Application Number 10/565,885, § 371(c) date: January 25, 2006, entitled "Administration of Anti-Cytokine $F(ab')_2$ Antibody Fragments."

2. I am currently employed at Laboratorios Silanes, S.A. de C.V. and Instituto Bioclon, S.A. de C.V. in México, D.F. I hold the position of Director de Investigación.

3. I received my education at the School of Chemistry and my posgraduated studies at the School of Medicine of the Universidad Nacional Autónoma de México (UNAM). A copy of my *curriculum vitae* is attached.

3. I am familiar with the above-identified application and pending claims as well as the Office Action dated July 31, 2009.

4. I understand that the present claims are directed toward methods for treating tumor necrosis factor-alpha (TNFα) mediated immune reaction that causes corneal transplant rejection, comprising topically administering directly to the eye of said patient an effective amount of anti-TNFα $F(ab')_2$ neutralizing antibody fragments.

5. I understand that claims 62, 64 and 65 were rejected in the Office Action dated July 31, 2009 as being obvious over Pluenneke (US 2001/0021380) in view of Fabrizio *et al.* (EP 0 492 448 A1), Horwitz (WO 92/22324), Adair *et al.* (EP 0 516 785 B1), and Reza Dana (WO 00/27421). I also understand that claim 63 was rejected in the Office Action dated July 31, 2009 as being obvious over Pluenneke (US 2001/0021380) in view of Fabrizio *et al.* (EP 0 492 448 A1), Horwitz (WO 92/22324), Adair *et al.* (EP 0 516 785 B1), Loorareesuwan *et al.*, (Am. J. Trop. Med. Hyg. 1999, 61:26-33), and Reza Dana (WO 00/27421). I have reviewed these references.

6. None of references cited by the Examiner provide experimental evidence for the effectiveness of anti-TNFα $F(ab')_2$ fragments in the treatment of corneal transplant rejection.

7. I supervised a longitudinal, double-blind study to test the effects of administration of anti-TNFα $F(ab')_2$ fragments to prevent rejection in cornea transplant. In this study, graft cornea were introduced into mice with nylon thread sutures using standard laboratory surgical techniques. Following cornea grafting, mice were treated for two weeks with quinolone in an eye drop formulation for 2 weeks (one drop, three times a day) and then treated with anti-TNFα $F(ab')_2$ fragments in an eye drop formulation, or placebo for 8 weeks (one drop, three times a day).

8. The rejection of graft cornea is associated with increased opacity of the donor tissue. The opacity of the graft cornea in treated mice was determined in 18 visits over 9 weeks using the following standard scale:

GRADE	OPACITY	OBSERVATIONS
0	Non-opaque	---
1	Minimal opacity	---
2	Medium opacity	Visible border of pupil and iris
3	Moderate opacity	Visible border of pupil only
4	Intense opacity	Visible anterior chamber
5	Critical opacity	Invisible anterior chamber

9. Figure 1 shows the cumulative survival of graft cornea (Y-axis) over the 18 visits (X-axis) in mice with critical opacity (grade 5). The probability of survival of cornea grafts for mice treated with anti-TNFα $F(ab')_2$ fragments (dashed line) at week 16 was 58% compared to 33% for mice treated with placebo (solid line), and a 57% increase in graft cornea survival was observed with anti-TNFα $F(ab')_2$ fragment treatment. These data show that treatment with anti-TNFα $F(ab')_2$ fragments significantly and unexpectedly increased graft cornea survival.

10. To further illustrate these results, I supervised additional experiments involving microphotography of cornea cross sections in mice treated with anti-TNFα $F(ab')_2$ fragments (Figure 2A) or with placebo (Figure 2B), one drop, three times a day for 8 weeks. Graft cornea from mice treated with placebo exhibited the following properties associated with graft rejection: neovascularization of the cornea, chronic granulomatous inflammation, lymphocyte infiltrates, anterior synechia (*i.e.*, adhesion) between the iris and crystalline lens, and intrastromal spaces surrounded by multinucleated giant cells (Figure 2B). In contrast, graft cornea from mice treated with anti-TNFα $F(ab')_2$ fragments exhibited the following properties: scarce lymphocyte infiltrates, scarce neovascularization, and integrity of the

anterior capsule (Figure 2A). Treatment with anti-TNFα $F(ab')_2$ fragments significantly and unexpectedly decreased the morphological properties of the cornea associated with graft rejection.

11. It is my opinion that the increased graft cornea survival and decreased morphological properties of the cornea associated with graft rejection would not have been expected in view of the art cited by the Examiner.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,

Dr. Jorge E. Pañiagua-Solís

Date: October 30th, 2009.

1040318

Curriculum Vitae

Jorge Fernando Paniagua-Solís

o DATOS PERSONALES

Fecha de Nacimiento: 20 de Julio de 1964

Lugar de Nacimiento: San Cristóbal de las Casas, Chiapas

Nacionalidad: Mexicana

Domicilio del Trabajo: Amores 1304, Col. Del Valle. C.P. 03100
México, D.F.
Tel: +52 55 5488-3751
Fax: +52 55 5604-7236

Posición Actual: Director de Investigación
Laboratorios Silanes, S.A. de C.V.
Instituto Bioclon, S.A. de C.V.
México, D.F.

Sistema Nacional de Investigadores:

Investigador Nacional Nivel I

Comisión Permanente de la FARMACOPEA de los Estados Unidos Mexicanos:

Nombramiento como Miembro Experto

Jorge Fernando Paniagua-Solís

ESCOLARIDAD

Doctorado en Ciencias Biomédicas-Inmunología- (1996). Facultad de Medicina de la U.N.A.M. Mayo 28, 1996

Maestría en Ciencias Biomédicas-Inmunología- (1990). Facultad de Medicina de la U.N.A.M. Abril 16, 1991

Químico Farmacéutico Biólogo-Bioquímico Microbiológico- (1983-1987). Facultad de Química, U.N.A.M. Junio 13, 1988

BECAS

Becario de Investigación Laboratorio de Inmunoquímica de la Unidad de Investigación Biomédica del C.M.N., I.M.S.S. (1988-1990)

Becario del Programa Universitario de Investigación en Salud, U.N.A.M. (1989-1993)

Becario del Gobierno Francés. Especialidad en Gestión de Tecnología (1994) París, Francia

IDIOMAS

Inglés

Francés

EXPERIENCIA PROFESIONAL

Investigador Asociado "D". Unidad de Investigación Médica en Inmunoquímica. Hospital de Especialidades. Centro Médico Nacional Siglo XXI. Instituto Mexicano del Seguro Social (1996-1998).

Investigador Asociado "B". Unidad de Investigación Médica en Inmunoquímica. Hospital de Especialidades. Centro Médico Nacional Siglo XXI. Instituto Mexicano del Seguro Social (1992-1996).

Jefe del Departamento de Investigación en Biológicos y Reactivos. Instituto Nacional de Higiene. Gerencia General de Biológicos y Reactivos. Secretaría de Salud (1990-1992).

Auxiliar Técnico. Laboratorio de Inmunoserología del Centro de Investigaciones Ecológicas de Sureste. San Cristóbal de las Casas, Chiapas (1981-1982).

o ACTIVIDADES DENTRO DE LA U.N.A.M.

Profesor de Asignatura "A" definitivo de Inmunología General. Facultad de Química (1994- a la fecha).

Profesor Titular del Diplomado en Inmunología Básica y su Aplicación al Laboratorio. Facultad de Química (1996).

Consejero Técnico Alumno Propietario. (1985-1987). Facultad de Química.

o PUBLICACIONES

⇒ Eliézer Martín Frías, Luis F. de Armas, **Jorge F. Paniagua Solís**, Boletín Sociedd Entomológica Aragonesa *Centruroides Gracilis (Latreille, 1804). Variabilidad de los Peines y descripción de algunas anomalías morfológicas (Scorpiones:Buthidae).*, pág.: 453-457, No. 44, 2009.

⇒ Susana Flores, Alejandro Alagón, Araceli Olguín, **Jorge Paniagua;** Innovación y Competitividad, *"Paquete tecnológico para la producción de un antiveneno polivalente contra la araña del género Loxosceles"* pág, 4-10, Núm 35 Julio-Septiembre 2009 **ISSN 1664-0123**

⇒ Blanca I. García-Gómez, TIMOTEO c. Olamendi-Portugal, **Jorge Paniagua,** Jurg ban der Walt, Karin Dyason, Lourival D. Possani; *"Heterologous expresión of a gene that cdes for pG8, a scorpio toxin of Parabuthus granulatus, capable of generating protecting antibodies in mice"*, **Toxicon 53 (2009): 770-778.**

⇒ Mata González, M.T., García Ubbelohde, W., Alagón Cano, A., Paniagua-Solís, J. **2008.** Anticuerpos monoclonales para uso terapéutico en humanos. **Informacéutico 15 (5):30-36.**

⇒ Estévez, J., Magaña, P., Chippaux, J-P., Vidal, N., Mancilla, R., **Paniagua, J.F.,** de Roodt, A.R. **2008.** Étude des venins des principaux serpents venimeux de Guyane française et de leur neutralisation. **Bull Soc Pathol Exot 101 (4) : 353-359.**

⇒ Martín-Frías, E., de Armas, L.F., **Paniagua-Solís, J. 2007.** Complementos a la taxonomía e historia natural de *Centruroides orizaba* Armas & Martín-Frías, 2003 (Scorpiones:Buthidae). **Boletín Sociedad Entomológica Aragonesa. 41:313-319.**

⇒ Sciutto, E., Morales, J., Martinez, J.J., Toledo, A., Villalobos, M.N., Cruz-Revilla, C., Meneses, G., Hernández, M., Díaz, A., Rodarte, L.F., Acero, G., Gevorkian, G., Manoutcharian, K., **Paniagua, J.,** Fragoso, G., Fleury, A., Larralde, R., De Aluja, A.S. and Larralde, C. **2007**. Further evaluation of the synthetic peptide vaccine S3Pvac against *Taenia solium* cysticercosis in pigs in na endemic town of México. **Parasitology. 134 (Pt1):129-33.**

⇒ Alejandro Olvera, Blanca Ramos-Cedillo, Judith Estévez, Herlinda Clement, Adolfo de Roodt, **Jorge Paniagua-Solís,** Hilda Vázquez, Alfonso Zavaleta, Maria Salas Arruz, Roberto P. Stock and Alejandro Alagón. **2006.** North and South American *Loxosceles* spiders: Development of a polyvalent antivenom with recombinant Sphingomyelinases D as antigens. **Toxicon 48:64-74.**

⇒ Ricardo Márquez-Velazco, Rafael Bojalil, Alma Buelna, Fernando Flores-Guzmán, Judith Estévez-Ramirez, Jorge Laguna, Ana M Hernández, Alberto Díaz-Quiñónez and **Jorge F. Paniagua-Solís. 2006**. Anti-tumor necrosis factor F(ab´)2 antibody fragments Project in murine polymicrobial sepsis: Concentration and early intervention are fundamental to the outcome. **Inflamm. Res. 55:378-384.**

⇒ Juan C. Almagro,Laisel Martínez, Sylvia L. Smith, Alejandro Alagín, Judtih Estévez, **Jorge Paniagua, 2006.** Analysis of the horse VH repertoire and comparison with the human IGHV germline genes, and sheep, cttle and pig VH sequences. **Mol Immunol 43 (11):1836-45.**

⇒ C. Hernández, J. Estévez-Ramírez, **Jorge F. Paniagua Solís.** Araña Vioinista: Problema médico en México. ISSN 1607-6788 Año 11, No. 6. **Junio 2006, Gaceta Biomédicas UNAM, Pág. 11.**

⇒ INVESTIGADORAS DE LA FM LOGRAN COMERCIALIZAR UNA PRUEBA RÁPIDA PARA LA DWETECCIÓN CUALITATIVA DE ANTICUERPOS CONTRA T. CRUZI EN SUERO O PLASMA (Patentes Universitarias) **Mayo 2006, Gaceta de la Facultad de Medicina UNAM No. 545.**

⇒ G. Sandoval, A. Olguín, **J. Paniagua; Diagnóstico de la Influenza Aviar;** ISSN1607-6788 Año 11, No. 5; **Mayo 2006, Gaceta Biomédicas UNAM, Pág. 11-12.**

⇒ I. Ochoa, J. Paniagua; **El sistema de patentes y el registro de los nuevos productos.** ISSN1607-6788 Año 11, No. 3; **Marzo 2006, Gaceta Biomédicas UNAM, Pág. 11**

⇒ A. Quiñones, **J. Paniagua., Participación de las organizaciones de investigación por contrato en el desarrollo de fármacos;** ISSN1607-6788 Año 11, No. 2; **Febrero 2006, Gaceta Biomédicas UNAM, Pág. 11.**

⇒ T. Mata-González, **J. Paniagua Solís., La industria naciente de los biogenéricos;** ISSN 1607-67-88, Año 11, No. 1; **Enero 2006, Gaceta Biomédicas UNAM**

⇒ V. Aguilar, **J. Paniagua;** Los comités de ética como base de la investigación biomédica. ISSN 1607-6788 Año 10, No. 12 **Diciembre de 2005 Gaceta Biomédicas UNAM, Pág. 5**

⇒ L. Uribe, L. Harrison, M. Parkhouse, A. Toledo, A. Fleury,. E. Sciutto, A. Olguin, **J. Paniagua Solís;** Prueba rápida para el diagnóstico oportuno de cisticercosis activa; ISSN 1607-67-88 Año 10, No. 11; **Noviembre de 2005; Gaceta Biomédicas UNAM, Pág. 11**

⇒ P. Magaña, J. Estévez, **J. Paniagua Solís,** El veneno de las serpientes y sus componentes, ISSN 1607-67-88 Año 10, No. 10; **Octubre de 2005; Gaceta Biomédicas UNAM.**

⇒ A. Buelna, A. Díaz, **J. Paniagua Solís;** validación de Inmunoensayos en la Industria Farmacéutica. ISSN 1607-67-88 Año10, No. 9; **Septiembre de 2005; Gaceta Biomédicas UNAM.**

⇒ H. Vázquez, **J. Paniagua Solís;** Toxinas recombinantes de arácnidos, una realidad, ISSN 1607-67-88 Año10, No. 8; **Agosto de 2005; Gaceta Biomédicas UNAM, Pág. 11.**

⇒ J. Estévez, **J. Paniagua Solís;** Sistemas de Calidad, ISSN 1607-67-88 Año 10, No. 6; **Junio 2005; Gaceta Biomédicas UNAM; Pág. 9.**

⇒ A. Díaz Quiñones; **J. Paniagua Solís;** Moléculas de la superfamilia del TNF; ISSN 1607-67-88 Año 10, No. 4; **Abril 2005; Gaceta Biomédicas UNAM, Pág. 11.**

⇒ F. Flores, A. Díaz Quiñones, **J. Paniagua Solís;** La utilidad de los cultivos celulares en la investigación, ISSN 1607-67-88 Año 10, No. 3; **Marzo 2005; Gaceta Biomédicas UNAM, Pág. 11.**

⇒ H. Vázquez, A.Chávez-haro, W. García-Ubbelohde, R. Mancilla-Nava, **J. Paniagua-Solís,** A. Alagón, C. Sevcik **2005.** Pharmacokinetics, of a F(ab')2 scorpion antivenom in healthy human volunteers. **Toxicon: 46 (7): 797-805**

⇒ De Roodt AR, Esteves-Ramirez J, **Paniagua-Solís JF,** Litwin S, Carvajal-Saucedo A, Dolab JA, Robles-Ortíz LE, Alagón A. **2005.** Toxicity of venoms from snakes of medical importance in México, **Gac Med Méx. 141(1):13-21**

⇒ Jessica Y. López-Correa, Eliézer Martín-Frías, Arturo Ramírez-Cruz, **Jorge F. Paniagua-Solís. 2005,** Judith Estévez Ramírez. Maduración Diverticular en *Diplocentrus tehuano* Francke 1977 (Scorpiones diplicentrinae) **Entomología Mexicana . 4:71-73**

⇒ Adolfo R. de Roodt, Oscar D. Salomón, Tomás A. Orduna, Luis E. Robles, **Jorge F. Paniagua Solís,** Alejandro Alagón C. **2005.** Envenenamiento por picaduras de abejas, **Gac Méd Méx.141(3):215-22.**

⇒ W. García; **J. Paniagua Solís;** Orígenes del VIH; ISSN 1607-67-88 Año 09, No. 12; **Diciembre 2004, Gaceta Biomédicas UNAM, Pág. 3.**

⇒ W. García, A. Díaz Quiñones, **J. Paniagua Solís;** Adoptando a las Enfermedades Huérfanas; ISSN 1607-67-88 Año 9, No. 9; **septiembre 2004, Gaceta Biomédicas UNAM, Pág 11.**

⇒ W. García, **J. Paniagua Solís,** Del Matraz a la Cabecera del Paciente; ISSN 1607-67-88 Año 9, No. 8; **Agosto de 2004, Gaceta Biomédicas UNAM**

⇒ J. Estévez, **J. Paniagua Solís,** Las dendrotoxinas y su uso práctico. ISSN 1607-67-88 Año 9, No. 7; **Julio 2004, Gaceta Biomédicas UNAM, Pág. 5.**

⇒ A. Alonso, A. Olguin, **J. Paniagua Solís,** La Sífilis, ISSN 1607-67-88 Año 9, No. 6, **junio 2004, Gaceta Biomédicas UNAM, Pág. 8**

⇒ F. Flores, Alberto Díaz, **J. Paniagua Solís,** Las interacciones neuro-endocrino-inmunológicas, ISSN 1607-67-88, Año 9. No. 5, **Mayo 2004, Gaceta Biomédicas UNAM, Pág. 8.**

⇒ W. García, **J. Paniagua Solís,** Células Gliales: más que tejido de sostén. ISSN 1607-67-88, Año 9, No. 4, **Abril 2004, Gaceta Biomédicas UNAM, Pág. 9.**

⇒ C. Hernández, J. Estévez, **J. Paniagua Solís,** Los venenos de serpientes, herramientas de la neurofisiología, ISSN 1607-67-88, Año 9. No. 3; **Marzo 2004, Gaceta Biomédicas UNAM, Pág. 9.**

⇒ D. Ferreiro, L. Uribe, A. Olguin, **J. Paniagua Solís,** Desarrollo de un sistema de diagnóstico para detección de envenenamiento por la araña café violinista. ISSN 1607-67-88, Año 9, No. 2; **Gaceta Biomédicas UNAM, Pág. 9.**

⇒ A. Díaz, **J. Paniagua Solís,** Esfuerzos de la Industria Farmacéutica para invertir en Investigación y Desarrollo; ISSN 1607-67-88, Año 9, No. 1; **Gaceta Biomédicas UNAM, pág. 13.**

⇒ W. García, **J. Paniagua Solís,** Salud por la Salud, ISSN 1607-67-88, Año 8, No. 12; **Gaceta Biomédicas UNAM, Pág. 4.**

⇒ P. Magaña, J. Estévez, **J. Paniagua Solís,** Componentes de venenos de animales ponzoñosos como agentes terapéuticos; ISSN 1607-67-88 Año 8, No. 11; **Noviembre 2003, Gaceta Biomédicas UNAM, Pág. 11.**

⇒ L. Robles, **J. Paniagua Solís,** La tecnología como apoyo a la solución del problema de salud pública de los accidentes por animales ponzoñosos: Redtox.org, ISSN 1607-67-88, Año 8, No. 10, **Octubre 2003, Gaceta Biomédicas UNAM, Pág. 10.**

⇒ L. Uribe, A. Olguín, **J. Paniagua Solís,** El Dengue, un problema de salud pública, ISSN 1607-67-88, Año 8, No. 9, **Septiembre 2003, Gaceta Biomédicas UNAM, Pág. 6.**

⇒ W. García, **J. Paniagua Solís,** La Diabetes Mellitus, ISSN 1607-67-88, Año 8. Núm 8. **Agosto 2003, Gaceta Biomédicas UNAM, Pág. 7.**

⇒ J. Estévez, **J. Paniagua Solís,** El veneno y sus bondades, ISSN 1607-67-88 Año 8, No. 7; **Julio 2003, Gaceta Biomédicas UNAM, Pág. 7.**

⇒ A. Díaz, **J. Paniagua Solís;** Las balas mágicas empiezan a encontrar blancos. ISSN 1607-67-88 Año 8 No. 6; **Junio 2003, Gaceta Biomédicas UNAM, Pág. 7.**

⇒ L. Uribe, A. Olguin, **J. Paniagua Solís;** Diagnóstico rápido de hepatitis C, ISSN 1607-67-88 Año 8, No. 5. **Mayo 2003, Gaceta Biomédicas UNAM, Pág.13.**

⇒ B. Antuna, **J. Paniagua Solís;** ¿Nuevos alimentos o nuevos mercados?. ISSN 1607-67-88 Año 8 No. 4; **Abril 2003, Gaceta Biomédicas UNAM, Pág. 13.**

⇒ J. Estévez, **J. Paniagua Solís,** ¿Qué son los escorpiones o alacranes?. ISSN 1607-67-88 Año 8, No. 3; **Marzo 2003, Gaceta Biomédicas UNAM, Pág. 4.**

⇒ J. Estévez, **J. Paniagua Solís,** Serpientes, Mitos y Creencias. ISSN 1607-67-88 Año 7, No. 11; **Noviembre 2002, Gaceta Biomédicas UNAM, Pág. 11.**

⇒ A. Quiñones, I. Vallano, **J. Paniagua Solís,** La interacción entre la academia y la industria, ISSN 1607-67-88 Año 7, No. 10, **Octubre 2002, Gaceta Biomédicas UNAM, Pág. 13**

⇒ J. Estévez, **J. Paniagua Solís,** Reactividad Cruzada del Faboterápico Coralmyn® Hacia Diferentes Venenos de Serpientes de Micrurus spp. ISSN 1607-67-88 Año 7, No. 7; **Julio 2002, Gaceta Biomédicas UNAM, Pág. 7**

⇒ W. García, A. Buelna, **J. Paniagua Solís,** Hepatitis B, Diagnóstico y tratamiento. ISSN 1607-67-88 Año 7, No. 6. **Junio 2002, Gaceta Biomédicas UNAM, Pág. 10.**

⇒ A. Díaz Quiñones, L. Uribe, **J. Paniagua Solís,** El renovable interés por el Ántrax; ISSN 1607-67-88 Año 7, No. 1. **Enero 2002, Gaceta Biomédicas UNAM, Pág. 11.**

⇒ De Roodt AR, **Paniagua-Solís JF**, Dolab JA, Estévez-Ramírez J, Ramos Cerrillo B, Litwin S, Dokmetjian JC, Alagón A. **2004.** Effectiveness of two common antivenoms for North, Central, and South American Micrurus envenomations. **J. Tox. Clin. Tox. 42(2):171-8**

⇒ Jessica Yadira López-correa, Eliézer Martín-frías, Hortensia Montellanos-Rosales, **Jorge F. Paniagua-Solís. 2004.** Estudio anatómico e Histológico del aparato reproductor de *Centuroides Limpidus Limpidus Karsch* 1980 (Scorpiones: Buthidae) **Entomología Mexicana. 3 :60-62**

⇒ Márquez R, Laguna J, Polvorilla V, Buelna A, Diáz-Quiñónez A, **Paniagua-Solís J**, Bojalil R. **2004.** Attenuating without Depleting TNFa Levels Significantly Protects Mice Against Sepsis Induced by Cecal Ligation and Puncture, **Immunology. 337-40 ISBN 88-758-7070-5**

⇒ Blanca Ramos-Cerrillo, Alejandro Olvera, George V. Odell, Fernando Zamudio, **Jorge Paniagua Solís,** Alejandro Alagón and Roberto P. Stock. **2004** Genetic and enzymatic characterization of Sphingomyelinase D isoforms from the North American fiddleback spiders *Loxosceles boneti* and *Loxosceles recluse* . **Toxicon 44: (5): 507-14.**

⇒ R.Otero, E Navío, F.A. Céspedes, M.J. Núnez, L. Lozano, E.R. Moscoso, C. Mantallana, N.B. Arsuza, J. García, D. Fernández, J.H. Rodas, O.J. Rodríguez, J. E. Zuleta, J.P. Gómez, M. Saldarriaga, J.C. Quintana, V. Núñez, S. Cárdenas, J. Barona, R. Valderrama, N. Paz, A. Díaz, O.L. Rodríguez, M.D. Martínez R. Maturana, L.E. Beltrán, M.B. Mesa, **J. Paniagua, E.** Flórez, W.R. Lourenco **2004.** Scorpion envenoming in two regions of Colombia. **Trans. R. Soc. Trop. Med. Hygiene. 98:742-50**

⇒ Adolfo R. de Roodt, Susana Isabel García, Carlos Gómez, Judith Estévez, Eduardo G. Gould, **Jorge F. Paniagua-Solís**, Alejandro Alagón, Jorge A. Dolab, Osvaldo Curcí **2004.** Antitoxinas y Antivenenos para uso Terapéutico. **Acta Toxicol. Argent. 12(2):29-41.**

⇒ Adolfo R. de Roodt, Judith Estévez, Jorge Adrián Dolab, Blanca Ramos-Cerrillo, Alejandro Olvera, **Jorge F. Paniagua**, Penélope Magaña, Silvana Litwin, María Salas, Alfonso Zavaleta, Alejandro Alagón, **2004.** Toxicity and Inmunological reactivity of two North American *Loxosceles* ("Brown Spider") Venoms. **(Manuscrito en preparación)**

⇒ Judith Estévez; Penélope Magaña; Nicolas Vidal; Rita Mancilla ; Alejandro Alagón; **Jorge F. Paniagua**; Jean-Philipe Chippaux; Adolfo R. de Roodt. **2004.** Usefulness of different antivenoms in the neutralization of toxic activities on the venom of the principal venomous snakes form guyanas. **(Manuscrito en preparación)**

⇒ Armas L. F., De; E. Martín-Frías; **J.F. Paniagua-Solís 2001.** Taxonomic comments about some Mexican scorpions of the genus *Centruroides (Scorpiones:Buthidae)*. **An. Esc. Nac. Cienc. Biol., Méx., 47(2):167-171.**

⇒ **Paniagua Solís, Jorge F.** Periódico Reforma, Sección-Tema *Ciencia: "Hepatitis C"*

⇒ **Paniagua Solís, Jorge F.** Reforma.com, Sección-Tema *Ciencia: "Hepatitis C"*

⇒ **Paniagua Solís, Jorge F.** Periódico Excélsior, Sección-Tema *Salud: "Hepatitis C"*

⇒ **Paniagua-Solís, Jorge F.**, Mancilla, Rita, Gonzalez, Carlos; Alagón Alejandro. **2001** From Srotherapy to Fabotherapy. ***J. Venom. Anim. Toxins*, vol. 7, (2):340-340. ISSN 0104-7930.**

⇒ Santamaría, H., Manoutcharian, L., Rocha, E., González, G., Govezensky, L. Uribe, L.I., Olguín, A. **Paniagua, J.** And Gevorkian, G. **2001.** Identification of Peptide Sequences Specific for Serum Antibodies

from Human Papillomavirus-Infected Patients Using. Phage Display Libraries. **Clin. Immunol. 101:296-302.**

⇒ Secundino Y., **Paniagua-Solís, J.**, Isibasi, A., Sánchez, J. **1998.** A cloning vector for efficient generation of Cholera Toxin B gene fusions for epitope screening. **Mol.Biotechnology 10:237-239.**

⇒ González, C.R., Noriega, F.R., Huerta, S., Santiago, A., Vega, M., **Paniagua, J.**, Ortiz-Navarrete, V, Isibasi, A. Levin, MM. **1998.** Inmunogenicity of a *Salmonella typhi* CVD 908 candidate vaccine strain expressing the major surface protein gp63 of *Leishmania mexicana mexicana*. **Vaccine 16:1043-1052.**

⇒ González-Canudas, J., Díaz-Valencia, J., Campos-Chirinos, A., **Paniagua-Solís J.**, Rangel-Frausto S., Castañón J., Isibasi, A. **1997.** Estudio por PCR de la expresión de regiones Vβ en células humanas de sangre periférica en pacientes con sepsis grave. **Avances en Biotecnología Moderna. 4:D62**

⇒ Secundino, V., **Paniagua Solís, J.**, Isibasi, A., Sánchez, J. **1997.** Diseño y construcción de un vector para la expresión de epitopos de la porina OmpC, en el extremo amino terminal de la subunidad B de la toxina del cólera. Avances en **Biotecnología Moderna 4:V53.**

⇒ **Paniagua-Solís, J.**, Sánchez, J., Ortiz-Navarrete, V., González, C.R., Isibasi, A. **1996.** Construction of CTB fusion proteins for screening of monoclonal antibodies against *Salmonella typhi* OmpC peptide loops. **FEMS Microbiol. Letters 141:31-36.**

⇒ **Paniagua-Solís, J.**, Martín-Orozco, N., Ortiz-Navarrete, V., Ramírez, G., González, C.R., Isibasi, A. **1995.** Predicted epitopes of *Salmonella typhi* OmpC porin are exposed on the bacterial surface. **Inmunol. Infect. Dis. 5:244-249.**

⇒ González, C.R., Isibasi, A., Ortiz-Navarrete, V., **Paniagua, J.**, García, J.A., Ramírez, A., Salvatierra, B., Tapia, R., Sepúlveda, J., Gutiérrez, G., Kumate, J. **1995.** Prevalence of antibodies against *Entamoeba histolytica* in Mexico measured by ELISA. **Epidemiol. Infect. 115:535-543.**

⇒ González, C.R., Mejía, M.V., **Paniagua, J.**, Ortiz-Navarrete, V., Ramírez, G., Isibasi, A. **1995.** Inmune response to porins isolated from *Salmonella typhi* in different mouse strains. **Arch. Med. Res. 26:S99-S103.**

⇒ Isibasi, A. **Paniagua, J.**, Rojo, M.P., Martín, N., Ramírez, G., González, C.R., López Macías, C., Sánchez, J., Kumate, J., Ortiz-Navarrete, V. **1994.** Role of porins from *Salmonella typhi* in the induction of protective inmunity. **Ann. N.Y. Acad. Sci. 730:350-352.**

⇒ Castellanos, L., Martín, N., Chávez, M.A., **Paniagua, J.**, Isibasi, A., Ortiz-Navarrete, V. **1994**. Identification of peptides from *Salmonella typhi* OmpC that bind H-2 molecules. **Arch. Med. Res. 25:55.**

⇒ Sánchez, J. Arias, C., Solórzano, R.M., González, R., **Paniagua, J.**, Isibasi, A., Kumate, J. **1993**. Ingeniería genética de la toxina del cólera y estudios sobre la relación estructura-función de enterotoxinas. **Rev. Fac. Med. UNAM 36:35-40.**

⇒ Gómez Priego, A., Cruz-Gutiérrez, L.E., **Paniagua-Solís, J.F.**, Ruenes-Meza, M.T. **1993**. DIG-ELISA test in onchochersiasis. Serum antibodies and probability of infection. **Arch. Med. Res. 24:353-359.**

⇒ González.C.R., Isibasi, A., Ortiz-Navarrete. V., **Paniagua, J.**, García, J.A., Blanco ,F., Kumate,J.**1993.** Lymphocytic proliferative response to outer-membrane proteins isolated from *Salmonella.* **Microbiol. Inmunol.37:793-799.**

⇒ Blanco, F., Isibasi, A, González, C.R.., Ortiz, V., **Paniagua, J.** Arreguín, C., Kumate,J. **1993**. Human cell mediated immunity to porins from *Salmonella typhi.* **Scan. J. Infec. Dis.25:73-80.**

⇒ García, J.A., Paez, R., **Paniagua, J.**, Isibasi, A., Kumate,J. **1992** . Biosafety status of Salmonella typhi porins. **Arch. Med. Res. 23:198.**

⇒ Isibasi, A., Ortiz-Navarrete, V., **Paniagua, J.**, Pelayo, R., González, C.R.; García, J.A., Kumate, J. **1992.** Active protection of mice against *Salmonella typhi* by inmunization with strain specific porins. **Vaccine 10:811-813.**

⇒ **Paniagua, J.**, García, J.A., López, C. R., González, C.R., Isibasi, A., Kumate, J. **1992.** Vacunas conjugadas contra infecciones bacterianas: Fiebre Tifoidea. **Salud Pública. Méx. 34:268-273.**

⇒ García, J.A.; **Paniagua J.**; Pelayo, R., Isibasi, A., Kumate, J. **1992.** Factores de virulencia de Salmonella typhi en relación al desarrollo de nuevas vacunas. **Salud Pública. Méx. 34:262-267.**

⇒ Agundis, C.; Isibasi, A.; Ortiz, V.; Reyes, J.L.; **Paniagua. J.;** Ramírez, A. y Kumate, J. **1990.** Obtención de anticuerpos monoclonales contra glicoproteínas de membrana externa de *Entamoeba histolytytica"* **Arch. Invest. Med. (Mex.) 21 (Suppl):15-22.**

⇒ Isibasi, A.; González, C.; Ortiz, V.; Muy, M.; **Paniagua, J.**; Blanco, F.; Pelayo, R.; Magdaleno, V.; Ramírez, A. y Kumate, J. **1990.** Seroepidemiología de la Amibiasis en la región norte de la República Mexicana. **Arch. Invest. Med. (Mex.) 21 (Suppl): 163-174.**

⇒ Pelayo,R.; Isibasi,A; **Paniagua, J.**; Ortiz, V.; Muy, M.; González, C.; Islas, S. y Kumate, J. **1989**. Elaboración de un Inmonuabsorbente para la Purificación de Porinas de *Salmonella Typhi*9,12,Vi:D. **Arch. Invest. Med. (Mex.) 20:279-286.**

⇒ **Paniagua, J.**; Isibasi, A; Pelayo, R.; Ortiz, V.; Muy, M.; González, C.; García, A.; Islas, S. y Kumate, J. **1989**. Anticuerpos monoclonales anti-lipopolisacárido de *Salmonella typhi* 9,12,d,Vi. Ensayo de protección de protección pasiva en un modelo murino de fiebre tifoidea. **Arch.Invest.Med.(Mex.) 20:315-320.**

⇒ Isibasi, Armando; Ortiz, Vianney; Vargas, Martín, **Paniagua, Jorge;** González, César; Moreno, José and Jesús Kumate, **1988**. Protection against **Salmonella typhi** Infection in Mice after Inmunization with Outer Membrane Proteins Isolated from **Salmonella typhi** 9,12,d,Vi. **Infect. Inmun.56:2953-2959.**

⇒ Isibasi, Armando; Ortiz Vianney; Vargas Martín; Tellez-Girón, Jacobo; **Paniagua Jorge** y Kumate Jesús. **1988. V.** Papel de las proteínas de membrana externa de *Salmonella typhi* en la inducción de protección contra la fiebre tifoidea, en un modelo murino. **Gaceta Médica de México.124:92-97.**

⇒ Gómez Priego, A., **Paniagua Solís,J.F.**, Rivas Alcalá, R.A., Ruenes Meza, M.T.**1985.** Successful application of the difusion-in-gel-enzyme-inked inmunosorbent assay (DIG-ELISA) in onchocerciasis. **Trans. Royal Soc. Trop. Med. Hygiene. 79:159-161.**

o PATENTES OTORGADAS

⇒ Pharmaceutical composition of F(ab')2 antibody fragments.
Inventores: Juan López de Silanes, Rita G. Mancilla-Nava, **Jorge F. Paniagua-solís.**
USPTO 7,485,303 BZ
Fecha: Febrero 3, 2009.

⇒ Pharmaceutical composition of $F(AB_2)$ fragments of antibodies and method for the preparation thereof.
Inventores: López de Silanes, Juan, Mancilla Nava, Rita and **Paniagua Solís, Jorge**
WO 02/068475 A2
U.S. Pant. 6,709,655 B2
Fecha: Marzo 23, 2004

⇒ Composición Farmacéutica de Fragmentos F(ab')2 de anticuerpos y un proceso para su preparación.
Inventores: López de Silanes, Juan; Mancilla Nava, Rita;
Paniagua Solís, Jorge
Expediente: PA/a/2003/007992
No. Patente Mexicana 230257
Fecha: Septiembre 04, 2003

⇒ Pharmaceutical composition of F(ab)2 fragments of antibodies and method for the preparation thereof.
Inventores: **Paniagua Solis, Jorge**; Mancilla Nava, Rita and López de Silanes, Juan.
No. de Patente Australiana: 2002237586
Fecha: Abril 5, 2007.

o SOLICITUD DE PATENTES

⇒ Anti-Inflamatory protein ophthalmic preparation
Inventores; López de Silanes,Juan; **Paniagua Solís, Jorge;** Díaz Quiñónez, Alberto
International Patent Appl. No. PCT/MX2007/000049
Fecha: Abril 3, 2006

⇒ Horse: Human Chimeric Antibodies
Inventores: Juan C. Almagro, Alejandro Alagon-Cano,
Jorge F. Paniagua-Solís, Sylvia L. Smith, Alvaro Velandia
PCT/US2006/042236
Fecha: Octubre 28,2005

⇒ Administration of anti-cytokine F(ab)2 antibody fragments
Inventores: Díaz Quiñonez, Alberto; Mancilla Nava, Rita, Silanes, Juan; **Paniagua Solís, Jorge**
International Patent Appl. No. PCT/IB03/02971
Fecha: Julio 25, 2003

o LIBROS EDITADOS

⇒ **Jorge F. Paniagua-Solís (Ed). 2007.** En los territorios de Scorpio. *Experiencias y recuerdos de un alacranólogo.* **ISBN 970-94592-0-2**

⇒ **Jorge F. Paniagua Solís**, Leopoldo Santos Argumedo, Vianney Ortíz Navarrete. **2005** Segunda Reunión de Diagnósticos, Enfermedades Infecciosas Emergentes, **ISBN 970-94592-0-1**

⇒ **Jorge F. Paniagua Solís**, Leopoldo Santos Argumedo, Vianney Ortíz Navarrete. **2005** Segunda Reunión de Diagnósticos, Enfermedades Infecciosas Emergentes, **ISBN 970-94592-0-1**

o CAPITULOS EN LIBROS

⇒ Flores-Guzman, F., Alagon-Cano, A., **Paniagua-Solís, J. 2007.** Independent Large-Scale Lots of Anti-thyroid-stimulating Hormone Monoclonal Antibody Manufactured *In vitro* Contain Indentical Quality. **International Proceedings of the 13th International Congress of Immunology: 235-238. ISBN 978-88-7587-379-0.**

⇒ Márquez R, Laguna J, Polvorilla V. Buelna A, Díaz-Quiñonez A. **Paniagua-Solís J**, Bojalil R. 2004 *Attenuating withourt Depleting TNF a Levels Signifacantly Protects Mice Against Sepsis Induced by Cecal Liagtion and Puncture*. **In: "Immunology 2004, Immunodeficiency, infectious diseases, immunomodulation, and vaccines". (Collection of Free Papers, 12th International Congress of Immunology and 4th Annual Conference of FOCIS), pp.337-40. Montreal, Canada: Medimond S.r.l., Bologna, ISBN-88758-7070-5.**

⇒ González Canudas, J., **Paniagua Solís, J.**, Isibasi Araujo, A. **1997. Las alteraciones Inmunológicas de la Sepsis. En: sepsis.** Academia Mexicana de Cirugía. México. pp. 14-16. ISBN 970-91878-0-5.

⇒ Isibasi, A., **Paniagua, J.F.**, González, C.R., López Macías, C.R., Martín, N., Ortiz, V. **1996. Porinas: Inmunógenos experimentales contra la fiebre tifoidea. En: "Vacunas. Fundamentos para su desarrollo".** Cabrera-Contreras, R., Gómez de León., P. Cravioto, A. (Eds.). UNAM/El Manual Moderno, S.A. de C.V., México. pp. 145-152.

⇒ Isibasi, A., Ortiz V., Moreno J., **Paniagua, J.**, Vargas, M., González, C. And Kumate, J. **1988. The role of Outer Membrane Proteins from Gram-negative Bacteria as Vaccine with special emphasis in typhoid fever. Monoclonal antibodies against *Salmonella typhi* porins. In: *Cell function* and *Disease*.** L. Todd; J. Jaz and L. Parker. (Ed.) Plenum, New York. pp. 281-192.

⇒ Isibasi, A., **Paniagua J.**, González, C.R., García, J.A., Kumate, J. **1992. "Vacunación contra la Fiebre Tifoidea". En: "Vacunas, Ciencia y Salud".** A. Escobar Gutiérrez y J.L. Valdespino-Gómez. (Eds.) Instituto Nacional de Diagnóstico y Referencia Epidemiológicos. Secretaría de Salud. México. pp. 317-325.

⇒ **Paniagua, J.**, Isibasi, A., garcía, J.A., González, C.R., Ortiz-Navarrete, V., Kumate, J. **1992. Porins as protective immunogens against typhoid fever. En: Typhoid fever: Strategies for the 90's.** T. Pang, C.L. Koh and S.D. Puthucheary (Eds.) World Scientific Publishing Co. Pte. LTD. Singapore and London. pp 65-70.

⇒ González, C.R., Isibasi, A., **Paniagua, J.**, García, J.A., Kumate, J. **1991. Avances recientes en vacunas contra enteropatógenos. En: Avances en Enfermedades Diarréicas y Desequilibrio Electrolítico.** Mota H.F. y Arboleda, R. (Eds.) Secretaría de Salud. México. pp. 39-60.

○ CURSOS Y ESTANCIAS INTERNACIONALES

⇒ **Gestión en Biotecnología.** Escuela Superior de Comercio y Administración. París, Francia. Julio 1994-Enero 1995.

⇒ **Analytical Methods for Proteins.** American Chemical Society Short Course. Chicago, IL., USA. Marzo de 1991.

⇒ F.E.B.S. International summer School on Immunology. **Genes, Receptors and regulation of the Immune system.** Spetsai, Grecia, Agosto-Septiembre de 1990.

⇒ **Estancia en Institute for Immunology and Genetics**, German Cancer Research Center, Heidelberg, Alemania, Septiembre de 1990.

o TESIS DIRIGIDAS

⇒ **Licenciatura:** Tamayo Sánchez Halina Lucero **"Liberación del Factor de Crecimiento Tisular beta (TGF-beta), en sujetos con Enfermedad Respiratoria Exarcebada por Aspirina (EREA)"**, Facultad de Química Universidad Nacional Autónoma de México, en México 03/2009.

⇒ **Licenciatura:** Eva Lidia Alvarado, **"Interacción de CD8-B7 y Moléculas Asociadas en la Activación de Linfocito T"**, Facultad de Química, Universidad Nacional Autónoma de México, en México, 10/1996.

⇒ **Licenciatura:** María del Carmen Montano Lechuga **"Obtención de un Anticuerpo Monoclonal Anti-Lipido A"**, Facultad de Estudios Superiores Zaragoza - Universidad Nacional Autónoma de México, en México 07/1996.

⇒ **Licenciatura:** Vicenta Cazares Domínguez **"Mapeo de Dos Epitopos de la Porina OmpC de Salmonella Typhi"**, Facultad de Química Universidad Nacional Autónoma de México, en México 10/1994.

⇒ **Licenciatura:** Luz María Mónica Fajardo, **"Obtención de Anticuerpos Monoclonales"** Facultad de Química - Universidad Nacional Autónoma de México, en México 10/1994.

o PREMIOS Y DISTINCIONES

⇒ Participación en el programa radiofónico **"Imagen en la ciencia, por pura curiosidad"** que se transmite por Radio IMAGEN 90.5 con el tema **"Antivenenos y Alacranismo en México"**

⇒ **Reconocimiento de la Asociación Mexicana de Directivos de la Investigación Aplicada y el Desarrollo Tecnológico, A.C. por el tercer lugar del Premio ADIAT a la Innovación Tecnológica 2009 en la categoría PyME con el caso: Paquete tecnológico para la producción de un antiveneno polivalente (Loxmyn) contra la araña del género Loxoceles.**

⇒ **Medalla por 15 años de servicios académicos en la UNAM 2009.**

⇒ **Medalla al Mérito Sancristobalense. 2006.** "Dr. Manuel Velazco Suárez".

⇒ **Premio Nacional de Tecnología 2005.** Otorgado al Instituto Bioclon, S.A. de C.V.

⇒ **Mención Honorífica Examen de Grado (Doctorado)** el 11 de Abril de 1996.

⇒ **Medalla "Gabino Barreda" por desempeño durante los estudios de la Maestría en Ciencias Biomédicas.**

⇒ **Mención Honorífica Examen de Grado (Maestría)** el 5 de Noviembre de 1990.

⇒ **Premio Nacional de la Juventud 1989.** Área de Actividades Productivas. Otorgado por la Presidencia de la República.

⇒ **Premio Anual de Investigación Médica.** "Dr. Jorge Rosenkrans, 1989". **"Anticuerpos Monoclonales anti-idiotipo: Modelo de Vacuna Murina contra *Salmonella typhi* 9,12Vi:d"** Instituto Syntex. México, D.F.

⇒ **2° Lugar. Concurso de la mejor Tesis del año.** Sociedad Mexicana de Bioquímica Clínica y Laboratorios AMES, 1989.

⇒ **Diploma de aprovechamiento por haber obtenido el 2° lugar en la carrera de QUIMICO FARMACEUTICO BIOLOGO durante el período escolar 1983-1987/1.** Otorgado por la UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO.

⇒ **Mención Honorífica. Examen Profesional** del 19 de Febrero de 1988.

o MEMBRESIAS

⇒ Miembro del Comité de Expertos de Biotecnología de la Farmacopea Mexicana.
⇒ Asociación Mexicana de Directivos de la Investigación Aplicada y el Desarrollo Tecnológico, A.C.
⇒ Asociación Farmacéutica Mexicana (2000)
⇒ Colegio Nacional de QFB's (2000)
⇒ International Society on Toxinology (1998).
⇒ Sociedad Mexicana de Biotecnología y Bioingeniería (1996)
⇒ Asociación de Cooperación México-Francia, A.C. (1995)
⇒ Sociedad Mexicana de Inmunología (1989).
⇒ Asociación Mexicana de Bioquímica Clínica (1989).

Figure 1: Cumulative Survival of Graft Cornea with Anti-TNFα $F(ab')_2$ Fragment Treatment

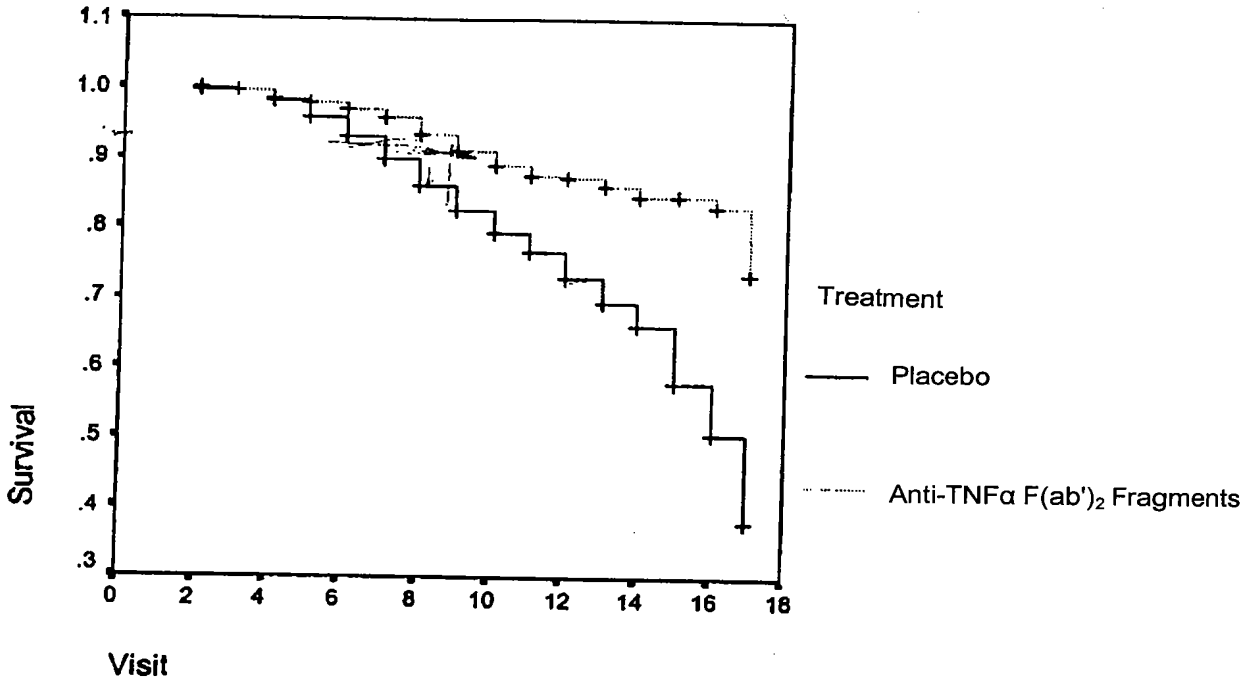
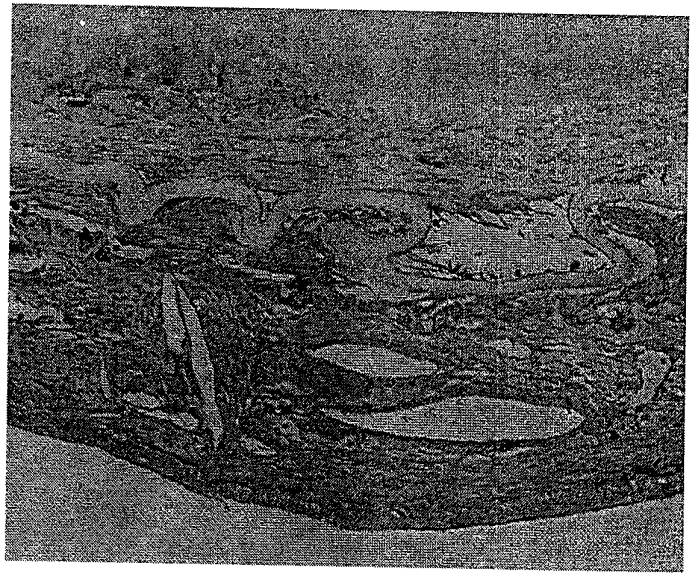
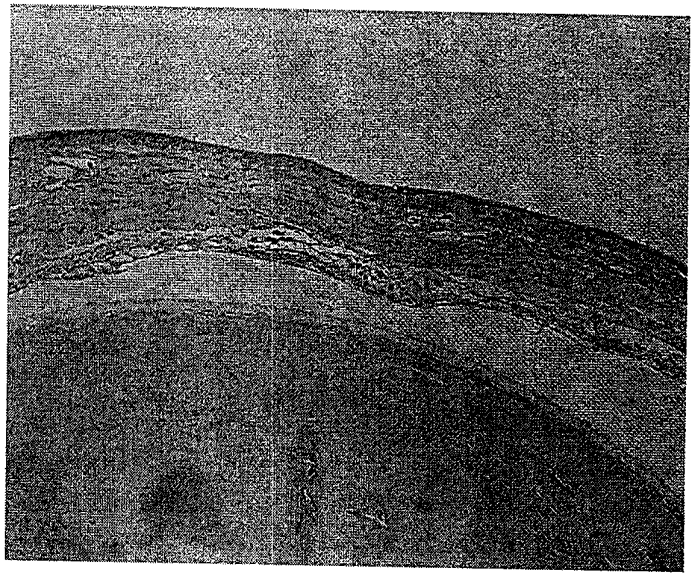


Figure 2: Graft Cornea Morphology with Anti-TNFα $F(ab')_2$ Fragment Treatment

A



B



Attachment to Exhibit F: Declaration Under 37 C.F.R. § 1.132 of Dr. Jorge F. Paniagua-Solís

The $F(ab)_2$ anti-TNFα antibody eye drop formulations used in the experiments of Figures 1 and 2 of Exhibit F contain the following components and concentrations:

Component	Range of concentration (mg/ml)
$F(ab)_2$ anti-TNFα antibody	0.01-50
NaH_2PO_4 analytic grade	5.0-50
Na_2HPO_4 analytic grade	30-80
NaCl analytic grade	0.5-10
Glycerin analytic grade	0.05-10
H_3BO_4 analytic grade	0.01- 20
Propylene glycol analytic grade	0.05-10
Water (injectable)	To reach 1 ml (final volume)

Observations of inflammatory cells in the cornea stroma related to Figure 2 of Exhibit F were performed using immunohistochemistry with the following antibodies and reported according to the number of cells found in two fields at 40X magnification.

Antibody	Origin	Concentration	Specificity	Conjugation	Free	Dilution	Provider
C-II			Mouse				SM Hybridoma CINVESTAV
SAv-HRP							CINVESTAV
$F(ab)_2$	Goat		Horse				CINVESTAV
TCR γδ	Hamster	0.5 mg/ml	Mouse	Biotin	(-)	1:100	Pharmingen
TCR αβ	Hamster	0.5 mg/ml	Mouse	Biotin	(-)	1:100	Pharmingen
TNFα	Rat	0.5 mg/ml	Mouse	(-)	(-)	1:100	Pharmingen
CD14	Rat	0.5 mg/ml	Mouse	(-)	(+)	1:100	Pharmingen
CD11c	Hamster	0.5 mg/ml	Mouse	FITC/PE	(-)	1:100	Pharmingen
CD19	Rat	0.5 mg/ml	Mouse	Biotin	(-)	1:100	Pharmingen

Abbreviations: SAv-HRP (streptavidin-horseradish peroxidase), C-II (class II), FITC (fluorescein-5-Isothiocyanate), PE (Phycoerythrin)